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# Acute and Chronic Toxicity of Chlordane to Fish and Invertebrates

Chemico Process Plants Co, El Monte, Calif Envirogenics Systems

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16. ABSTRACT

The acute and chronic toxicity of technical chlordane to bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), brook trout (Salvelinus fontinalis), Daphnia magna, Hyallela azteca, and Chironomus No. 51 were determined with flow-through conditions. The purpose was to estimate concentrations producing acute mortality and those having no effect on the long-term survival, growth, and reproduction of the various species. Whole body residues of technical chlordane components were measured in the three invertebrate species at the end of the chronic exposure tests.

Concentrations of technical chlordane causing 50% mortality in 96 hr were 36.9 μg/l for fathead minnow, 47 μg/l for brook trout, and 59 μg/l for bluegill, while that causing 50% immobilization in the cladoceran, D. magna, was 28.4 μg/l. The amphipod, H. azteca, was only slightly affected at 96 hr by the chlordane concentrations tested, and the 168-hr EC50 was 97.1 μg/l. Acute mortality of midges, Chironomus No. 51, was not successfully evaluated.

With respect to the test conditions employed and life cycle stages evaluated, the lowest concentrations of technical chlordane found to cause major chronic effects were 0.32 μg/l for brook trout, 1.22 μg/l for bluegill, 1.7 μg/l for midges, 11.5 μg/l for amphipods, and 21.6 μg/l for cladocerans

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| Trout | Residues | Amphipod | |
| Minnows | | Midge | |
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# ACUTE AND CHRONIC TOXICITY OF CHLORDANE TO FISH AND INVERTEBRATES

by

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# FOREWORD

Our nation's freshwaters are vital for all animals and plants, yet our diverse uses of water---for recreation, food, energy, transportation, and industry--physically and chemically alter lakes, rivers, and streams. Such alterations threaten terrestrial organisms, as well as those living in water. The Environmental Research Laboratory in Duluth, Minnesota, develops methods, conducts laboratory and field studies, and extrapolates research findings.

--to determine how physical and chemical pollution affects aquatic life

--to assess the effects of ecosystems on pollutants

--to predict effects of pollutants on large lakes through use of models

--to measure bioaccumulation of pollutants in aquatic organisms that are consumed by other animals, including man

This report describes the acute and chronic effects of the pesticide chlordane on a number of freshwater fishes and invertebrates.

Donald I. Mount, Ph.D.
Director
Environmental Research Laboratory
Duluth, Minnesota

# ABSTRACT

The acute and chronic toxicity of technical chlordane to bluegill (Lepomis macrochirus), fathead minnow (Pimephales promelas), brook trout (Salvelinus fontinalis), Daphnia magna, Hyallela azteca, and Chironomus No. 51 were determined with flow-through conditions. The purpose was to estimate concentrations producing acute mortality and those having no effect on the long-term survival, growth, and reproduction of the various species. Whole body residues of technical chlordane components were measured in the three invertebrate species at the end of the chronic exposure tests.

Concentrations of technical chlordane causing 50% mortality in 96 hr were 36.9 μg/l for fathead minnow, 47 μg/l for brook trout, and 59 μg/l for bluegill, while that causing 50% immobilization in the cladoceran, D. magna, was 28.4 μg/l. The amphipod, H. azteca, was only slightly affected at 96 hr by the chlordane concentrations tested, and the 168-hr EC50 was 97.1 μg/l. Acute mortality of midges, Chironomus No. 51, was not successfully evaluated.

With respect to the test conditions employed and life cycle stages evaluated, the lowest concentrations of technical chlordane found to cause major chronic effects were 0.32 μg/l for brook trout, 1.22 μg/l for bluegill, 1.7 μg/l for midges, 11.5 μg/l for amphipods, and 21.6 μg/l for cladocerans.

Technical chlordane accumulation in the invertebrate species varied directly with the aqueous concentration to which the animals were exposed. The component accumulated to the greatest extent was trans-nonachlor, for which whole body residues were up to 145,000-times higher than the aqueous concentration.

This report was submitted in fulfillment of Contract No. 68-01-0187 by the Chemico Process Plants Company-Envirogenics Systems under the sponsorship of the U.S. Environmental Protection Agency. Work was completed as of June 1974.

# CONTENTS

Foreword. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . iii
Abstract. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . iv
Tables. . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . vi
List of Abbreviations and Symbols . . . . . . . . . . . . . . . . . ix
Acknowledgments . . . . . . . . . . . . . . . . . . . . . . . . . . x

I Introduction. . . . . . . . . . . . . . . . . . . . . . . . . . 1
II Conclusions.. . . . . . . . . . . . . . . . . . . . . . . . . . 3
III Recommendations . . . . . . . . . . . . . . . . . . . . . . . . 4
IV Literature Review . . . . . . . . . . . . . . . . . . . . . . . 6
V Materials and Methods . . . . . . . . . . . . . . . . . . . . . 21
VI Results . . . . . . . . . . . . . . . . . . . . . . . . . . . . 34
VII Discussion. . . . . . . . . . . . . . . . . . . . . . . . . . . 76

Literature Cited. . . . . . . . . . . . . . . . . . . . . . . . . . 81
Bibliography. . . . . . . . . . . . . . . . . . . . . . . . . . . . 87
Appendix Tables . . . . . . . . . . . . . . . . . . . . . . . . . . 90

# TABLES

No. Page

1 Concentrations of Chlordane Toxic to Fish .......... 10

2 Concentrations of Chlordane Toxic to Aquatic Invertebrates .......... 16

3 Characteristics of Fish Exposed to Technical Chlordane in Acute Toxicity Tests .......... 22

4 Water Quality During Acute Toxicity Tests of Technical Chlordane .......... 35

5 Measured Concentrations of Technical Chlordane in Acute Toxicity Tests .......... 37

6 Total Lengths of Fathead Minnow Fry Chronically Exposed to Technical Chlordane .......... 40

7 Lengths and Weights of Adult Fathead Minnows at Termination of Chronic Exposure to Technical Chlordane .......... 41

8 Mortality of $F_o$-Generation Fathead Minnows During Chronic Exposure to Chlordane .......... 43

9 Spawning History of Fathead Minnows Chronically Exposed to Technical Chlordane .......... 44

10 Mortality and Relative Size of $F_1$-Generation Fathead Minnows Chronically Exposed to Technical Chlordane .......... 45

11 Growth of $F_o$-Generation Bluegill During Chronic Exposure to Technical Chlordane .......... 47

| No. | | Page |
|---|---|---|
| 12 | Mortality of $F_o$-Generation Bluegill During Chronic Exposure to Technical Chlordane | 49 |
| 13 | Spawning History of Bluegill Chronically Exposed to Technical Chlordane | 50 |
| 14 | Conditions of Adult Bluegill at Termination of Chronic Toxicity Test | 52 |
| 15 | Survival of $F_1$-Generation Bluegill in Chronic Toxicity Test of Technical Chlordane | 54 |
| 16 | Growth of $F_1$-Generation Bluegill During Chronic Toxicity Test of Technical Chlordane | 55 |
| 17 | Total Lengths of $F_o$-Generation Brook Trout Chronically Exposed to Technical Chlordane | 57 |
| 18 | Body Weights of $F_o$-Generation Brook Trout Chronically Exposed to Technical Chlordane | 58 |
| 19 | Mortality of $F_o$-Generation Brook Trout Chronically Exposed to Technical Chlordane | 59 |
| 20 | Spawning Success of Brook Trout Chronically Exposed to Technical Chlordane | 60 |
| 21 | Viability and Hatch of Embryos and Conditions of $F_1$-Generation Brook Trout Alevins | 61 |
| 22 | Growth of $F_1$-Generation Brook Trout During Chronic Exposure to Technical Chlordane | 63 |
| 23 | Relative Survival and Growth of *Hyallela azteca* Exposed to Technical Chlordane | 65 |
| 24 | Contents and Concentration Factors (C.F.) of Chlordane Constituents in Dried *Hyallela azteca* That Had Been Exposed to Technical Chlordane | 67 |

| No. | | Page |
|---|---|---|
| 25 | Survival and Reproduction of *Daphnia magna* in Chronic Toxicity Test of Technical Chlordane ........ | 69 |
| 26 | Average Dry Body Weights of First Instar *Daphnia magna* Produced During Fourth Week of Chronic Toxicity Test of Technical Chlordane ........ | 71 |
| 27 | Contents and Concentration Factors (C.F.) of Chlordane Constituents in Dried *Daphnia magna* That Had Been Exposed to Technical Chlordane ........ | 73 |
| 28 | Chronic Effects of Technical Chlordane on *Chironomus* No. 51 ........ | 74 |

# LIST OF ABBREVIATIONS AND SYMBOLS

## ABBREVIATIONS

LC50 -- median lethal concentration
EC50 -- median effective concentration
MATC -- maximum acceptable toxicant concentration
A.I. -- active ingredient
LC100 -- lethal concentration to all test organisms
LC0 -- lethal threshold concentration
LT50 -- median lethal time
C.F. -- concentration factor

## SYMBOLS

$\hat{\sigma}$ -- logarithm of the standard deviation of the population tolerance frequency distribution
S -- antilogarithm of $\sigma$
mg/l -- milligram per liter
μg/g -- microgram per gram
ppb -- parts per billion = microgram per kilogram or microgram per liter

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# SECTION I

# INTRODUCTION

The organochlorine insecticide, chlordane, is widely used for the control of insect pests, particularly in non-agricultural areas. The insecticide has been extensively used relative to other organochlorine insecticides; in 1971, over 11.4 x $10^6$ kg of chlordane was produced compared to 5 x $10^4$ kg endrin, dieldrin and lindane, 4.5 x $10^6$ kg aldrin, and 21 x $10^6$ kg DDT (1). Because this chemical has a high biological potency and is relatively long-lived in the environment (2), it presents a potential hazard to non-target species (fish and wildlife) and ultimately to public health.

The adverse effects of chlordane on fish and aquatic invertebrates can be examined with acute and chronic toxicity tests or with studies of the accumulation of toxic (to predatory animals including man) residues (3). All of these methods have limitations (4), but the chronic toxicity test, which encompasses all or most of one reproductive cycle, probably represents the most direct method of estimating the concentration of chlordane which is "safe" for long-term survival, growth, and production of a species.

Less than a decade ago, water quality criteria for aquatic organisms were set with application factors ranging from 1/10 to 1/100 or with various equations (5, 6). These factors were multiplied by an acute toxicity test result such as the concentration lethal to 50% of the test specimens (LC50) to estimate environmentally "safe" concentrations. But these application factors were arbitrary and presented the hazard of over or underestimating the "safe" level. Because of the need to adequately protect the aquatic resource with realistic standards, other methods were examined. The chronic toxicity test has been suggested as the most practicable tool for achieving objective evaluations of sublethal, long-term toxicant effects on many species. The concept of the chronic test, its methods, scope, and application, was initially set forth by Mount and Stephan (7) in their studies of malation and the butoxyethanol ester of 2,4-D using fathead minnows (*Pimephales promelas* Rafinesque). Basically, the chronic test attempts to estimate the toxicant concentration at which effects on survival, growth and reproduction of all life stages become statistically indistinguishable from those for fish held in uncontaminated water (controls). Between the concentration producing some effect on one or more of the above indices and that having no effect is the maximum acceptable toxicant concentration (MATC), the theoretical "just safe" level. By dividing the MATC estimate by the LC50, an application factor can be obtained which can be used to estimate "safe" levels for aquatic organisms which are unsuitable for chronic toxicity testing for one reason or another. Several chronic toxicity tests of pesticides have been completed since that of Mount and Stephan (7). These include evaluations of malathion (8), carbaryl (9), and captan (10). Although application factors may remain relatively constant for a particular chemical and taxonomic group of organisms, the constraints on its applicability need to be defined. It has been recently shown, that the MATC and application factors can be satisfactorily estimated for at least one heavy metal through the use of one rather than a multiple generation test (11).

As discussed in the next section, a moderate amount of information is already available on the toxicity of chlordane to aquatic life. However, the majority of the tests have been conducted with static conditions for short periods and without measurement of chlordane concentrations in the diluent water. While these tests suffice to define the approximate order of chlordane toxicity to aquatic biota, their limited experimental scope restrains, in most cases, their use in establishing water quality criteria. To our knowledge, no chronic toxicity, reproductive studies of chlordane have heretofore been reported in the literature, and these studies are generally thought to be necessary for establishing sound standards. Accordingly, this investigation sought to define the acute and chronic toxicity of chlordane to three species of freshwater fish and three invertebrates using flow-through conditions. Furthermore, measurements of chlordane residues in tissues of the chronically exposed invertebrates were incorporated into the experimental design to provide information on the extent of bioaccumulation and on the potential hazard to predator species from consuming animals contaminated with this insecticide.

## SECTION II

## CONCLUSIONS

1. The acute mortality tests suggested that technical chlordane was a cumulative poison, causing toxicity as a function of concentration and exposure time. Median lethal thresholds were not attained within 96 hr for any species. The fathead minnow was the only species for which a threshold was observed, and it did not occur until approximately 180 hr.

2. Technical chlordane was generally more toxic on a chronic sublethal basis to the three fish species than to the three species of invertebrates.

3. Chronic toxicity test results suggest that technical chlordane concentrations greater than approximately 0.3 μg/l would be deleterious to the production of at least some fish species, and that concentrations greater than 21.6 μg/l would probably be very deleterious to most aquatic animals.

4. Accumulation of technical chlordane in the cladoceran and the amphipod was substantial, but varied with respect to the component. *Cis*-nonachlor was concentrated to the greatest extent and heptachlor the least. Residues of technical chlordane were not detected in the midge.

5. Measured concentrations of technical chlordane were consistently less than desired, even though low concentrations of the non-ionic surfactant, Triton X-100, and of the solvent, acetone, were employed to aid dissolution, and the toxicant solutions were continuously replenished. This indicates that toxicity tests of this insecticide would not be valid unless based upon measured concentrations of the dissolved compound.

# SECTION III

# RECOMMENDATIONS

1. Acute toxicity tests of technical chlordane should be continued until a median lethal threshold is observed rather than discontinued at a specified time. This would permit characterization of the toxicity curve particular to each species and life stage, and might prove useful in calculating effects in mixing zones.

2. Additional acute and chronic toxicity tests of technical chlordane using additional species of freshwater fish (e.g. Ictaluridae, Catastomidae), marine fish (e.g. Cyprinodontidae, Clupeidae), freshwater and marine invertebrates, and algae are needed, regardless of whether water quality standards are set for groups of aquatic organisms inhabiting specific ecosystems or on the average MATC of the most sensitive groups. Such information is needed to permit objective decisions on what levels of this insecticide will have no effect on diverse communities of aquatic organisms since there may or may not be important differences between taxonomic groups and between freshwater and marine organisms.

3. The acute and chronic toxicities of technical chlordane in mixtures of other toxicants and with different environmental conditions should be determined to evaluate interactions.

4. A multiple generation chronic toxicity test should be conducted to determine whether this complex insecticide causes teratogenic or mutagenic effects. For freshwater fish a species which completes its life cycle rapidly, such as the flagfish (Jordanella floridae Goode and Bean) or a poeciliid (e.g. the mosquitofish, Gambusia affinis [Baird and Girard]), should be considered since they have relatively short generation times of 3 to 4 months.

5. Studies of technical chlordane contents in fish and invertebrates should incorporate investigations of the uptake, biotransformation, and tissue distribution of each of the major, and perhaps the minor, constituents (e.g. hexachlorocyclopentadiene).

7. The degree and efficiency of transfer of technical chlordane components through several trophic levels should be determined and compared to uptake from the water.

8. The dosage levels of technical chlordane causing lethal and sublethal effects on predators should be determined by feeding them prey species contaminated with known amounts of the insecticide.

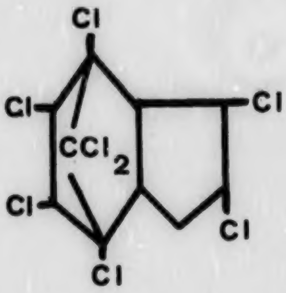
9. Increasing the number of replicates per treatment for the $F_0$-generation from the presently recommended two to at least three and possibly four would considerably strengthen the value and power of statistical tests.

# SECTION IV

# LITERATURE REVIEW

## CHEMISTRY OF CHLORDANE

Chlordane (1, 2, 4, 5, 6, 7, 8, 8-octachloro-2, 3, 3a, 4, 7, 7a-hexahydro-4, 7-methanoindene) is a chlorinated hydrocarbon insecticide manufactured by the Velsicol Chemical Corporation (Chicago).

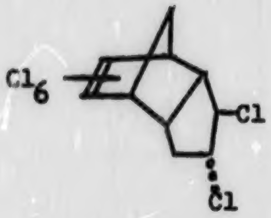


**Chlordane**

The technical grade used in the present program was supplied by Velsicol and identified as "Analytical Reference Technical Chlordane." It is a complex mixture and has been variously characterized by Velsicol (12), the U.S. Environmental Protection Agency (EPA, 13), and Saha and Lee (14). The predominant constituents are *trans*-chlordane (24 ± 2%), *cis*-chlordane (19 ± 3%), heptachlor (10 ± 3%), chlordenes (20.5%), *trans*-nonachlor (5.1%), and *cis*-nonachlor (2.8%) (page 7 and Appendix Table 1).

Technical chlordane is a liquid with a molecular weight of approximately 410. Its solubility limit in the laboratory water used in these investigations was of the order of 150 to 220 µg/l at 22°C. Edwards (15) has given its solubility as 100 µg/l at 20 to 30°C. A typical chromatogram of the technical material is shown in Fig. 1. Identification was accomplished with known standards and EPA (13) data.

The stability of technical chlordane solutions in distilled water has been evaluated by Bevenue and Yeo (16) for a period of 60 days. The authors



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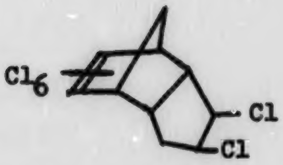
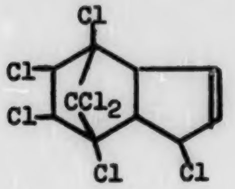


cis-chlordane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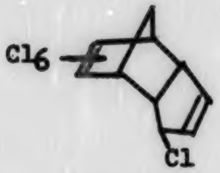

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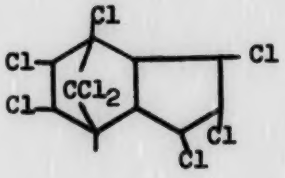
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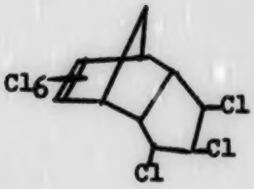
nonachlor



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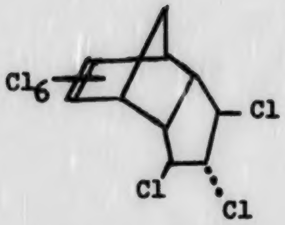
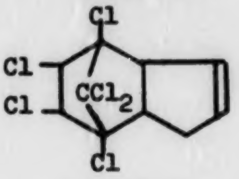


trans-nonachlor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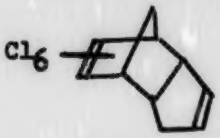

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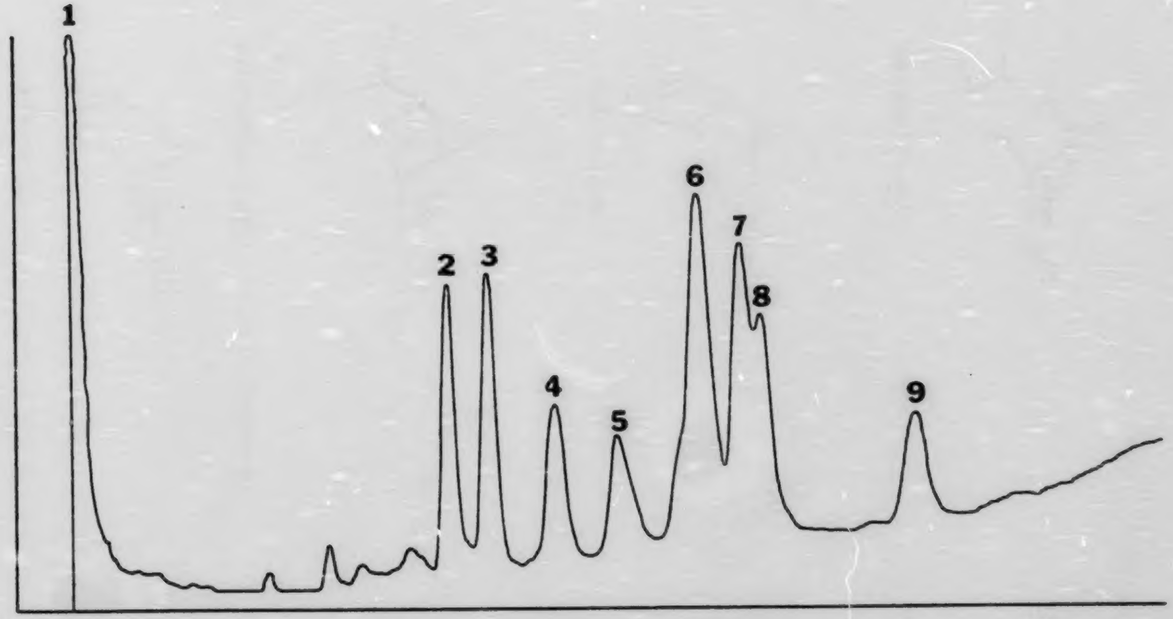

Fig. 1. Chromatogram of technical chlordane standard in hexane run under conditions specified elsewhere: (1) n-hexane; (2) $C_{10}H_6Cl_6$ isomer; (3) heptachlor and chlordene; (4) $\gamma$ - $\beta$ and "A"-chlordene; (5) $C_{10}H_7Cl_6$ isomer; (6) *trans*-chlordane; (7) *cis*-chlordane; (8) *trans*-nonachlor; (9) *cis*-nonachlor.

noted no temporal changes in trans- (γ) chlordane and cis- (α) chlordane, or the nonachlor components, but observed a conversion of heptachlor to 1-hydroxychlordene. There was no generation of heptachlor epoxide, and the occurrence of oxychlordane was not mentioned. Octachlor epoxide, also known as oxychlordane, is formed by the epoxidation of chlordane and is a known metabolite of chlordane in animals (17, 18).

## TOXICITY OF CHLORDANE TO AQUATIC ANIMALS

Although chlordane has received far less study than such chlorinated hydrocarbons as DDT and endrin, a substantial volume of literature exists concerning its toxicity to aquatic animals. The majority of the literature concerns the insecticide's efficacy in killing mosquitoes (Diptera: Culicidae).

### Fish

Chlordane is generally less acutely toxic to fish than endrin, DDT, dieldrin, and aldrin, and more toxic than lindane and methoxychlor, for example. Much of the available literature on chlordane toxicity to fish is summarized in Table 1. The majority of these toxicity tests were conducted for less than 96 hr and, except for two, employed static test conditions. In preparing Tables 1 and 2, efforts were made to convert test results, where possible, to concentrations equivalent to 100% active ingredient (A.I.) for direct comparison. As can be seen, responses to chlordane ranged from 0.1 μg/l A.I., which significantly increased oxygen consumption in bluegill (19) to 3,050 μg/l A.I., a level lethal to rainbow trout exposed to an emulsion of chlordane in a flow-through system (20).

Comparison of the toxicity test results conducted with methodology similar or identical to the standard procedures set forth by Doudoroff et al. (5) and the American Public Health Association (APHA, 21) lessens the disparity in reported toxic concentrations. Henderson, Pickering and Tarzwell (22) exposed four species of freshwater fish to an enulsifiable concentrate of 75% chlordane and found 96-hr LC50 values which varied from 16.5 μg/l (bluegill) to 142.5 μg/l (guppy, *Poecilia reticulata*). The concentrate was also more toxic to fathead minnows in soft water than in hard. During the same year, Clemens and Sneed (24) reported that 500 μg/l chlordane produced 50% mortality in channel catfish (*Ictalurus punctatus*) fingerlings in 96 hr. The later data of Katz (23) for three species of salmonids and the euryhaline stickleback (*Gasterosteus aculeatus*) and of Macek, Hutchinson, and Cope (25) for bluegill were in closer aggreement with the data of Henderson et al. (22) than with that of Clemens and Sneed (24). Ninety-six hour median lethal concentrations for the three salmonids ranged from 44 to 57 μg/l (23). The study of Macek et al. (25) indicated that chlordane was more toxic to bluegill at higher water temperatures than at lower ones. At higher temperatures successively less chlordane was required to produce 50% mortality in 24 hr. After 96 hr bluegill were killed less rapidly at 12.7°C (LC50 of 85 μg/l) than at 18.3°C (LC50 of 70 μg/l), but they were killed at the same concentration between 18.3°C and 23.8 °C.

## TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] µg/l | Type of response | Reference |
| Chinook salmon | *Oncorhynchus tshawytscha* | 96 | 57.0 | LC50[b] | 23 |
| Coho salmon | *O. kisutch* | 96 | 56.0 | LC50 | 23 |
| Rainbow trout | *Salmo gairdneri* | 96 | 44.0 | LC50 | 23 |
| Threespine stickleback | *Gasterosteus aculeatus* | 96 | 90[c] | LC50 | 23 |
| Threespine stickleback | *Gasterosteus aculeatus* | 96 | 160[d] | LC50 | 23 |
| Fathead minnow | *Pimephales promelas* | 96 | 39[e] | LC50 | 22 |
| Fathead minnow | *Pimephales promelas* | 96 | 52[f] | LC50 | 22 |
| Bluegill | *Lepomis macrochirus* | 96 | 16.5 | LC50 | 22 |
| Goldfish | *Carassius auratus* | 96 | 61.5 | LC50 | 22 |

Continued .....

## TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] µg/l | Type of response | Reference |
| Guppy | *Poecilia reticulata* | 96 | 142.5 | LC50 | 22 |
| Channel catfish | *Ictalurus punctatus* | 96 | 500 | LC50 | 24 |
| Bluegill | *L. macrochirus* | 96 | 85 (12.7 C) | LC50 | 25 |
| Bluegill | *L. macrochirus* | 96 | 77 (23.8 C) | LC50 | 25 |
| Murrel | *Channa punctatus* fry | 115 | 0.25 | LC100[g] | 26 |
| Murrel | *Channa punctatus* fingerling | 50 | 1.25 | LC100 | 26 |
| Murrel | *Channa punctatus* adult | 60 | 16.0 | LC100 | 26 |
| Rohu | *Labeo rohita* fingerling | 40 | 0.025 | LC100 | 26 |
| Spiny eel | *Mastocembelus pancalus* | 51 | 0.4 | LC100 | 26 |

Continued ....

## TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] µg/l | Type of response | Reference |
| Tengra | *Mystus vittatus* | 60 | 0.5 | LC100 | 26 |
| Nandus | *Nandus nandus* | 25 | 0.63 | LC100 | 26 |
| Punti | *Puntia sophore* | 18 | 1.25 | LC100 | 26 |
| Singhi | *Heteropneustes fossilis* | 51 | 1.25 | LC100 | 26 |
| Cuchia | *Amphipnous cuchia* | 45 | 2 | LC100 | 26 |
| Carp | *Cyprinus carpio* (embryos) | 91 | 3,600 | signif. effect on hatching | 27 |
| Bluegill | *L. macrochirus* | 30 | 200 | Lethal | 28 |
| Largemouth bass | *Micropterus salmoides* | 87 | 200 | Lethal | 28 |
| Rainbow trout | *S. gairdneri* | 24 | 3,050[h] | LC50 | 20 |
| Bluegill | *L. macrochirus* | 24 | 218[i] | LC50 | 29 |

Continued ....

TABLE 1. CONCENTRATIONS OF CHLORDANE TOXIC TO FISH--continued

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] μg/l | Type of response | Reference |
| Bluegill | *L. macrochirus* | 24 | 346[j] | LC50 | 29 |
| Bluegill | *L. macrochirus* | ? | 0.1 | Increased $O_2$ consumption | 19 |
| Carp | *C. carpio* | 48 | 1,160 | LC50 | 30 |
| Rainbow trout | *S. gairdneri* | 24 | 600 | Threshold conc. | 30 |
| Rainbow trout | *S. gairdneri* | 48 | 10 | LC50 | 6 |
| Pike | *Esox* sp. | 24 | >5 | Threshold conc. | 30 |
| White mullet | *Mugil curema* | 24 | 43 | LC50 | 31 |
| White mullet | *Mugil curema* | 48 | 5 | LC50 | 31 |

[a] All chlordane concentrations were adjusted, where possible, to 100% active ingredient.

[b] Median lethal concentration.

[c] Salinity was 5 g/kg.

[d] Salinity was 25 g/kg.

[e] Hardness of 20 mg/l $CaCO_3$.

[f] Hardness of 400 mg/l $CaCO_3$.

[g] Concentration lethal to all test specimens.

[h] LC50 calculated from concentration - % mortality data of authors.

[i] Chlordene (isomers of technical chlordane).

[j] Photochlordene (photolytic degradation product of chlordene).

In 1962, Lüdemann and Neumann (30) published extensive data on the toxicity of insecticides to a variety of fish, invertebrates, and a species of toad (Bufo bufo). The chlordane concentrations at which mortality just began to occur (i.e. lethal thresholds) for carp (Cyprinus carpio), rainbow trout (Salmo gairdneri), and pike (Esox sp.) exposed for 24 to 48 hr were 400, 600, and 5 μg/l, respectively. The 48-hr LC50 for carp was 1,160 μg/l. One of the more recent reports on chlordane toxicity dealt with the insecticide's lethality to several species of fish from India. Konar (26) found chlordane to have a uniformly high toxicity, but gave LC50 values without stating the time required for manifestation of the responses. LC100 estimates, concentrations lethal to 100% of the fish, ranged from a 115-hr value of 0.25 μg/l for murrel fry (Channa punctatus) to a 60-hr value of 16.0 μg/l for murrel adults. The 24-hr LC50 value of 10 μg/l chlordane for rainbow trout reported by the National Technical Advisory Committee on Water Quality Criteria (6) was one-sixtieth of the lethal threshold value reported by Lüdemann and Neumann (30) and one-fourth of that reported by Katz (23) for rainbow trout exposed for 96 hr. Although such wide variation among laboratory toxicity test results reinforces the need for standardization of toxicity testing procedures, measurement of the actual concentrations of pesticide to which the organisms were being exposed rather than reliance on the amount added to the tanks would probably have narrowed the range in values.

Little information was found documenting the toxicity of chlordane to marine organisms. Katz (23) exposed stickleback to chlordane at salinities of 5 and 25 g/kg and observed the pesticide to be approximately half as toxic at the higher salinity (96-hr LC50 of 160 μg/l) as at 5 g/kg (96-hr LC50 of 90 μg/l). In one of the few acute toxicity tests conducted with a flow-through rather than a static system, Holden (31) reported 24- and 48-hr LC50 values for juvenile white mullet (Mugil curema) of 43 and 5.5 μg/l, respectively.

The lowest concentration of chlordane (0.1 μg/l) found to elicit a potentially deleterious response (i.e. an increase in oxygen consumption) was observed by Dowden (19) in studies of the effects of various insecticides on bluegill. Augmented metabolic requirements of chlordane-exposed fish were also observed by Malone and Blaylock (27) in evaluations of the toxicity of DDT, chlordane, dieldrin, endrin, diazinon, and O, O, dimethyl-S-(4-oxobenzotriazino-3-methyl) phosphorodithioate to carp embryos. Concentrations of chlordane below 720 μg/l A.I. shortened incubation and stimulated embryonic development. Twenty-three percent of the chlordane-treated embryos hatched after 52.5 hr and 71% after 69.5 hr. None of the control embryos hatched by 52.5 hr and only 54.7% by 69.5 hr. Koch, Cutkomp and Yap (32) reported chlordane inhibition of $Mg^{++}$-ATPase activity in both mitochondrial and non-mitochondrial preparations of bluegill brain tissue. Since ATPase converts ATP to ADP and inorganic phosphate, inhibition of this enzyme would uncouple oxidative phosphorylation and thereby stimulate metabolism.

Little research has been performed on behavioral responses of fish to chlordane. Summerfelt and Lewis (33) reported that 5, 10 and 20 mg/l concentrations of a 75% emulsifiable concentrate of chlordane repelled green sunfish (Lepomis cyanellus), that 2 mg/l would result in an equivocal response, and that 1 mg/l would produce no response. Since these levels are considerably greater than lethal levels, it appears that fish encountering an acutely lethal concentration of this insecticide might be unable to detect and avoid it.

There have been several studies of the effects of chlordane introduced into natural watercourses. Using flow-through conditions, Cope, Gjullin and Storm (20) introduced acetone solutions and emulsions of chlordane into troughs and streams containing principally salmonids, caddisflies (Trichoptera) and blackflies (Diptera-Simuliidae) in experiments designed to determine whether concentrations effective in controlling the simuliids would be deleterious to populations of salmonids and their prey. Emulsions of 1,250 µg/l A.I. chlordane immobilized trout in 15 min and caused death within 24 hr in tests conducted in troughs. A median lethal concentration of 3,050 µg/l was calculated from the data of Cope et al. (20) for rainbow trout exposed to chlordane for 15 min and held for 48 hr in uncontaminated water. In stream tests an emulsion of 1,250 µg/l chlordane immobilized the trout in 15 min. In a later study, Mulla (34) assessed the toxicity of various insecticidal preparations, including an emulsifiable concentrate of chlordane, to mosquitofish (*Gambusia affinis*) and bullfrogs (*Rana catesbeiana*), both predators of mosquitos. Applied at 0.23 kg/acre (0.5 lb/acre), chlordane was moderately toxic to mosquitofish, but at 0.45 kg/acre (1.0 lb/acre), it was highly toxic. Bullfrog mortality was judged moderate to severe at applications of 0.23 kg/acre of the emulsifiable concentrate. Dosages for insect control are generally recommended to be less than 0.45 kg/acre.

## Aquatic Invertebrates

A considerable amount of work has been performed on the toxicity of chlordane to aquatic invertebrates. Owing perhaps to non-standardization of test conditions and use of different response criteria, water quality, and specimens of varying conditions, there is considerable disparity in the test results summarized in Table 2. Toxicities ranged from a 25-hr LC100 of 0.33 µg/l for backswimmers, *Notonecta* sp. (26), to a 96-hr lethal threshold of 10,000 µg/l for mussels, *Dreissena polymorpha* (30). Acute sensitivities of invertebrates were generally of the same order as those for fish.

The two most extensive studies on chlordane toxicity to invertebrates were performed by Konar (26) and Lüdemann and Neumann (30). Konar (26) exposed nine species of aquatic insects resident in India to chlordane for up to 168 hr and reported lethal threshold (LC0), LC50, and LC100 concentrations. However, exposure times varied within the 168-hr maximum and were not given for the LC50 values, thus limiting the latter's usefulness. As noted above, Konar (26) found the backswimmer to be the most sensitive species and the water scorpion, *Nepa* sp., the least sensitive (90-hr LC100 of 78.8 µg/l). Lüdemann and Neumann's (30) work encompassed more taxonomic groups than that of Konar (26). Lethal thresholds encountered with 24- to 96-hr exposure periods ranged from 1 µg/l for the amphipod, *Carinogammarus ruesilii*, to 10,000 µg/l for *D. polymorpha*. The lethal thresholds for the

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] μg/l | Type of response | Reference |
| Backswimmer | *Notonecta* sp. | 25 | 0.33 | LC100[b] | 26 |
| Water stick | *Ranatra filiformis* | 110 | 1.0 | LC100 | 26 |
| Water scorpion | *Nepa* sp. | 90 | 78.8 | LC100 | 26 |
| Water bug | *Sphaerodema annulatum* | 68 | 1.25 | LC100 | 26 |
| Giant water bug | *Belostoma indica* | 88 | 1.25 | LC100 | 26 |
| Aquatic beetle | *Hydrophilus* sp. | 130 | 1.58 | LC100 | 26 |
| Aquatic beetle | *Dytiscus* sp. | 90 | 2.0 | LC100 | 26 |
| Aquatic beetle | *Cybister* | 112 | 1.25 | LC100 | 26 |
| Dragonfly | Suborder anisoptera | 132 | 1.0 | LC100 | 26 |
| Non-biting midge | *Chironomus* (larvae) | 8 | 15 | LT50[c] | 36 |

Continued ....

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES--continued

| Species name | | Response manifest at | | | |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] µg/l | Type of response | Reference |
| Brine shrimp | *Artemia salina* (*nauplii*) | 2-3 | 10 | LT50 | 37 |
| American oyster | *Crassostrea virginica* | 24 | 10 | Inhibit growth | 38 |
| Caddisfly | *Hydropsyche* sp. | 34 | 1,650[d] | LC50 | 20 |
| Amphipod (scud) | *Gammarus lacustris* | 96 | 26 | LC50 | 35 |
| Stonefly | *Pteronarcys californica* | 48 | 55 | LC50 | 6 |
| Water flea | *Simocephalus serrulatus* | 48 | 55 | EC50[e] | 6 |
| Tubificid worm | *Tubifex tubifex* | 96 | 1,000 | Lethal threshold | 30 |
| Mussel | *Dreissena polymorpha* | 96 | 10,000 | Lethal threshold | 30 |

Continued ....

TABLE 2. CONCENTRATIONS OF CHLORDANE TOXIC TO AQUATIC INVERTEBRATES--continued

| Species name |  | Response manifest at |  |  |  |
|---|---|---|---|---|---|
| Common | Binomial | time, hr | conc.,[a] µg/l | Type of response | Reference |
| Amphipod (scud) | *Carinogammarus ruesilii* | 24 | 1 | Lethal threshold | 30 |
| Copepod | *Cyclops strenuus* | 24 | 1,000 | Lethal threshold | 30 |
| Isopod | *Asellus aquaticus* | 24 | 50 | Lethal threshold | 30 |
| Crayfish | *Cambarus affinis* | 24 | 1,000 | Lethal threshold | 30 |
| Diptera (Culicidae) | *Corethra plumicornis* (larvae) | 24 | 100 | Lethal threshold | 30 |
| Diptera (Chironomidae) | *Chironomus* (larvae) | 24 | >5 | Lethal threshold | 30 |

[a] All chlordane concentrations were adjusted, where possible, to 100% active ingredient.

[b] Lethal concentration to all specimens.

[c] Median lethal time.

[d] LC50 calculated from concentration - % mortality data of authors.

[e] Median effective concentration (EC50) is the concentration causing immobilization of the test specimens.

amphipod and for larval midges, *Chironomus* sp. (i.e. 5 μg/l), were similar to results obtained by others for species within the same taxonomic groups. Sanders (35) determined that the 96-hr LC50 for the amphipod, *Gammarus lacustris*, was 26 μg/l, while Silvey (36) calculated a median lethal time of 8 hr for *Chironomus* larvae exposed to 15 μg/l. The National Technical Advisory Commission on Water Quality Criteria (6) reported that the 48-hr LC50 and EC50 values for Stoneflies (*Pteronarcys californica*) and a cladoceran (*Simocephalus serrulatus*) were 55 and 20 μg/l, respectively.

Very little data have been accumulated on the toxicity of chlordane to marine invertebrates. Michael, Thompson and Abramowitz (37) determined that 10 μg/l would cause 50% mortality in brine shrimp nauplii (*Artemia salina*) in 2-3 hr. Butler, Wilson and Rick (38) investigated the effects of various insecticides on behavior and growth of Eastern oysters, *Crassostrea virginica*, and observed inhibition of growth within 24 hr at 10 μg/l chlordane.

Field experiments of chlordane toxicity have dealt primarily with the control of mosquitoes. Although these data will not be discussed herein, selected references are given in the appended Bibliography (Section IX). In an Alaskan field study of the adverse effects of insecticide applications for blackfly eradication on native populations of fish and aquatic insects, Cope et al. (20) found the caddisfly, *Hydropsyche* sp., to be the most sensitive of three species evaluated. Fifteen minute exposure to 1,250 μg/l chlordane immobilized *Hydropsyche* sp. Calculation of an LC50 for *Hydropsyche* sp. using concentration-percent mortality data given by Cope et al. (20) resulted in a value of 1,650 μg/l. The exposure consisted of a 15-min period of insecticide contact followed by 24-hr confinement in flowing freshwater.

## Residues of Chlordane in Aquatic Organisms and the Environment

Following its introduction into the environment, chlordane probably shares the same fates as many of the chlorinated hydrocarbon insecticides. Some of the components of the technical material will vaporize and be carried away from the point of application by thermal convection, etc. The vapor pressure of chlordane is $1 \times 10^{-5}$ mm Hg, more than that of DDT ($1.9 \times 10^{-7}$) and endrin ($2 \times 10^{-7}$); hence, its volatilization might be greater than these two compounds (15). Terrestrially, chlordane can be absorbed by organisms, adsorbed to particulate matter (soil), or enter watercourses by dissolving in water or sorbing to suspended particles. Chlordane is a relatively persistent compound in the environment, a characteristic that contributes to its bioactivity. Lichtenstein and Polivka (2) found that 12.4 to 17.8% of the chlordane applied to turf plots remained after 12 yr in undisturbed sandy loam soil. Heptachlor, a constituent of technical chlordane, had disappeared completely after 9 yr in silty clay loam soil. In comparison, Miami silt loam and muck soils treated with 10 and 100 lb/acre DDT retained approximately 22 and 33% of the insecticide after 3.5 yr (39). Thus, chlordane is persistent relative to other organochlorine pesticides in soil.

Stability to chemical, physical, and biological degradation is one of the prerequisites for a chemical to be available for uptake by organisms (bioaccumulation) and accumulated and transferred up food chains (biomagnification). There are several reports in the literature indicating that chlordane is accumulated, but no studies were found documenting biomagnification of this pesticide. Godsil and Johnson (40) found that DDT, chlordane and endrin, when applied seasonally during the summer growing season, would not accumulate in the aquatic food chain over successive years, but rather would decrease during winter to the limits of detection in both water and the biota. During the growing season, however, when the water contained up to 0.100 μg/l chlordane, algae (Cladophora sp.) were found to contain up to 50 ppb chlordane; vascular plants (Myriophyllum sp. and Potamogeton sp.), to 67 ppb; chubs (90% Siphateles bicolor and 10% S. gila), to 24 ppb; and clams (Gonidea sp.), to 12.0 ppb. Four different groups of largemouth bass and two groups of clams were also held for varying periods in cages in the same stream in which the natural populations of plants and animals were sampled. Largemouth bass accumulated from 8 to 43 ppb chlordane in less than 120 days, whereas accumulation by clams was 2 to 25 ppb (40). In a far more extensive pesticide monitoring program, Henderson, Johnson and Inglis (41) collected and analyzed 62 species of fish from the Great Lakes and major U.S. river basins for nine pesticides and their metabolites. Roughly 128 of the 587 composites of fish sampled (21.8%) contained detectable residues of chlordane. Whole body contents ranged to 7.3 ppm. The Gulf Coast fish contained the highest incidence of chlordane (45.8%) and the Columbia River system the least (1.7%). Green et al. (42), in a survey of 109 sites in U.S. rivers, stated that aqueous concentrations of chlordane in situ were only 0.1 μg/l.

Although the oceans can be regarded as an ultimate depository for a proportion of the chlordane applied on the mainland, a recent study by Duke and Wilson (43) on the contents of insecticides in 29 species of marine fish from the West Coast of the United States revealed no detectable residues of chlordane. While this finding might imply that chlordane's stability and susceptibility to biomagnification are less than those of DDT and its metabolites, for which residues up to 1,026 ppb were measured by Duke and Wilson (43), analytical methods for DDT and its metabolites are generally much more sensitive than those for chlordane and may, in fact, obscure chlordane on a chromatogram (personal communication, L. Mueller, EPA, Environmental Research Laboratory--Duluth). Hence, results of pesticide residue surveys in situ may not necessarily reveal the extent of chlordane biomagnification in natural populations of aquatic organisms.

# SECTION V

# MATERIALS AND METHODS

## ACUTE TOXICITY TESTS

### Test Species

Fish--The acute lethal toxicity of technical chlordane was examined using brook trout, *Salvelinus fontinalis* (Mitchill), bluegill, *Lepomis macrochirus* Rafinesque, and fathead minnow. Yearling brook trout were obtained from the California State Department of Fish and Game, held for 1 year, and tested as adults. Bluegill were obtained as juveniles from a local commercial dealer, while fathead minnow were reared in the laboratory from stock that had been originally obtained from the U.S. Environmental Protection Agency's Environmental Research Laboratory (ERL-D) at Duluth, Minnesota. The ages and sizes of the fish used for testing are given in Table 3.

Invertebrates--The crustacean, *Daphnia magna* Straus (Branchiopoda, Cladocera), was obtained from ERL-D and cultured in both continuous-flow and static systems. The non-biting midge, *Chironomus* No. 51[1] (Diptera, Chironomidae), was obtained from the University of California's Department of Entomology at Riverside, and the amphipod or "scud", *Hyallela azteca* (Saussure), was collected from small, constant temperature (16°C) streams immediately adjacent to the California Department of Fish and Game's Fillmore Hatchery.

### Acclimation and Toxicity Testing Conditions

All acute toxicity tests were conducted in accordance with methods recommended by The Committee on Methods for Toxicity Tests with Aquatic Organisms (44) and Sprague (45).

The fish were acclimated to toxicity test conditions for at least 2 months under controlled conditions. Brook trout and bluegill were fed a dry pelleted ration (Moore-Clark Co., Salt Lake City, Utah), the former at 2% of their body weight per day and the latter *ad libitum* twice daily. Fathead minnow fry were fed a mixture of 50% brine shrimp nauplii, 25% dry trout starter (Moore-Clark), and 25% "TetraMin" (Tetra-Werke, West Germany), *ad libitum* twice daily. Three days prior to conducting an acute toxicity test, 10 fathead minnow, 10 bluegill, or 5 brook trout were randomly distributed

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[1] The species has not been described, but has been classified in the interim as No. 51 by the University.

TABLE 3. CHARACTERISTICS OF FISH EXPOSED TO TECHNICAL CHLORDANE IN ACUTE TOXICITY TESTS

| Species | Approximate age at testing, months | Developmental stage | Density in test chamber, g fish/l | Total length mm | Wet body weight g |
|---|---|---|---|---|---|
| Brook trout | | | | | |
| Test 1 | 24 | A[a] | 32.8 | 233[b]<br>±14 | 131<br>±26 |
| Test 2 | 24 | A | 43.0 | 248<br>±15 | 172<br>±36 |
| Fathead minnow | 3 | J[c] | 0.11 | 26.2<br>±4.5 | 0.18<br>±0.10 |
| Bluegill | 3 | J | 0.41 | 50.8<br>±6.4 | 1.85<br>±0.75 |

[a] Adult.

[b] Mean ±1 standard deviation.

[c] Juvenile.

into each of 12 randomly positioned test chambers. The orders of tank placement were established with a random numbers table. The large size of the brook trout necessitated use of smaller sample numbers. Unfortunately, much smaller trout or larger test chambers, though highly desirable, were unavailable at the time these tests had to be carried out. Test chambers were constructed of glass and silicone rubber cement (Dow-Corning). Those utilized for the brook trout and fathead minnow tests measured 30.5 x 30.5 x 30.5 cm, with water depths of 21.5 cm (20 l) and 17.7 cm (16.5 l), respectively. Bluegill were exposed in 91.4 x 30.5 x 30.5 cm glass chambers containing 4.25 l of water at a depth of 15.2 cm. For all three species, 2-l proportional diluters (46) supplied each of the 12 test chambers, which comprised five toxicant concentrations and a control in duplicate. Each diluter supplied sufficient water to replace 10 tank volumes per day, a rate which insured 90% molecular replacement in 6 hr. Toxicant concentrations were successively diluted by a factor of 0.75. A syringe dosing device delivered microliter amounts of the toxicant at each diluter cycle. During the 72-hr acclimation to test conditions and the 96-hr and 192-hr toxicant exposure periods, the fish were not fed. A photoperiod of 16 hr was employed in all experiments. Light intensity from fluorescent lamps (Sylvania "Gro-Lux" and Durotest "Optima") averaged 1010 lux (lu). Black plastic curtains were used to shield the fish from disturbance. Water temperatures in tests with bluegill and fathead minnow were thermostatically regulated at 25°C in air-conditioned rooms. Those with brook trout were similarly controlled at 15°C. Although water flow into the tanks maintained dissolved oxygen concentrations above 70% of air saturation in most tests, artificial aeration was required in those using trout to meet this requirement.

Dead fish were measured for total length to the nearest millimeter and, after excess moisture had been removed with toweling, for wet body weight to the nearest gram or milligram, depending on size. All fish were measured from the six treatments constituting one replicate. The data were later pooled when no size differences between treatments were detected. Length-weight measurements were not taken prior to toxicity testing since it was believed that the stresses associated with handling and anesthesia (47, 48, 49) would have a greater influence on the LC50 than the changes in body weights during the course of the test.

*Daphnia magna* were cultured with both static and flow-through conditions at room temperature (20° to 21°C). Those held in the static system were fed once daily and those in the flow-through system twice daily. The ration consisted of a blended and sieved (100 mesh) mixture of dried baker's yeast (4.5%), pelleted fish food (91%) and alfalfa (4.5%) in water. Second to third instars were used for testing, and they were not fed during toxicant exposure.

The amphipod, *H. azteca*, was cultured in a flow-through system at 16°C and fed pre-soaked aspen leaves, supplemented with dry pelleted fish food. Live *Myriophyllum* sp. was also introduced as a possible food supply and habitat. Juveniles (~ 5 mm in total length) were used for acute toxicity testing and were not fed during the test.

The sensitivity of Chironomus No. 51 to technical chlordane was not evaluated successfully in an acute toxicity test because a satisfactory means of testing was not found. Use of a substrate of fine sand, the preferred habitat of this species in the laboratory, would not permit temporal examination of the status of the test specimens without causing considerable disturbance, whereas preliminary trials indicated that confinement of the larvae in egg incubation cups resulted in a random, anomalous mortality.

Cladocerans and amphipods used for acute toxicity testing were acclimated to test conditions, i.e., photoperiod and temperature, but not to the specific test apparatus prior to introduction into the chambers. Shortly before chlordane exposure, 10 specimens were randomly distributed into each of the randomly positioned test chambers. The test chambers consisted of glass cylinders, 6.5 cm inside diameter (i.d.) and 7.5 cm long, suspended in 30.5 x 30.5 x 30.5 cm glass chambers. Nylon screen ("Nitex", 500 µ openings) was attached to one end of the cylinder with silicone rubber cement (Dow-Corning) to permit circulation and retain the specimens. Each of the large chambers contained two cylinders and was supplied with appropriate test concentrations from a 2-l proportional diluter (46). Chlordane solutions were delivered in microliter amounts to the diluter's mixing (M-1) cell with the syringe dosing device described by Mount and Brungs (46). Toxicant concentrations were successively diluted by 25%. The tests with D. magna lasted 96 hr. The test utilizing H. azteca was extended to 168 hr since mortalities were minimal within 96 hr, even though the highest calculated chlordane concentration tested approximated the solubility limit.

The response criterion of immobilization was satisfied when the specimens lay motionless on the bottom and did not move when gently prodded.

## Water Quality

The diluent water for all tests was supplied from local wells and was unchlorinated except for treatment of storage reservoirs for algal control 1 day per week. Total residual chlorine was not detected upon periodic measurement (leucocrystal violet method of APHA [21]), even on the day of chlorination (Friday). As a precautionary measure, however, activated carbon filters were installed on the line supplying the D. magna, Chironomus No. 51, bluegill, and fathead minnow acute and chronic tests. Due to high flow requirements (76,000 l/day) of several brook trout chronics being conducted concurrently, the associated high cost of dechlorination equipment, and the belief that there was a low probability of a chlorine toxicity problem, no activated charcoal filters were installed on this line which supplied all trout as well as the amphipod tests. As will be seen, mortalities believed due to residual chlorine were observed near the end of this research project in approximately 20- to 40-day trout alevins, but not in older or younger trout or in the other species.

Seven water quality variables were monitored routinely during each test: water temperature, dissolved oxygen concentration, pH, total alkalinity, acidity, total hardness, and specific conductance. Except for water temperature, which was recorded continuously, water quality in the test chambers was measured 24 hr prior to and at least once during chlordane addition for comparison of the effects of the toxicant's presence on water quality, as a check on the water's suitability for uncompromised organism survival, and for estimation of water quality variation. All variables were determined with standard methods (21, 50). Except for rare instances, measurements were made on samples collected less than 4 to 6 hr earlier.

A number of ions were also determined by a commercial laboratory to gain a more complete description of the water's composition. Calcium, magnesium, potassium, sodium, chloride, and sulfate ions were determined every 4 months over 1 yr, ammonia was measured biannually and the other compounds once (Appendix Table 2).

## CHRONIC TOXICITY TESTS

### Fathead Minnows

The design, apparatus and conditions employed for the chronic toxicity tests using fathead minnows were developed by the U.S. Environmental Protection Agency (51). The basic apparatus consisted of a 2-l proportional diluter which supplied successively diluted (by a factor of 0.5) concentrations of technical chlordane to twelve 42.5-l (91.4 x 30.5 x 30.5 cm) glass tanks, comprising five toxicant concentrations and a control in duplicate. Daily flow through each chamber averaged six tank volumes, assuring 90% molecular replacement in approximately 9 hr. After spawning commenced, two glass chambers (28.5 x 14 x 15 cm) were placed into one end of each adult tank for rearing the progeny. All three chambers were supplied separately with test water, although effluent from the fry chambers passed directly through screening into the tank containing the adults.

The test was conducted with a photoperiod regulated to produce gonadal recrudescence and senescence at a cycle simulating that existing at Evansville, Indiana. Sunlight was simulated with Durotest "Optima" and Sylvania "Glo-Lux" fluorescent bulbs and the intensity averaged 1010 lu.

The water temperature was 21°C upon initiation of the chronic test, but was raised within the first 8 weeks to 25°C and maintained at that temperature thereafter.

At the beginning of the chronic toxicity test, 5-day-old fathead minnow fry were randomly distributed into one fry chamber (50 specimens each) in each of the 12 adult chambers. The fish were initially fed Oregon Moist Pellet starter mash (Moore-Clark) and "TetraMin" tablets crushed to a fine powder. Since growth and survival were poor, the diet was replaced after 2 months with one of frozen brine shrimp nauplii. As the fish grew, they were fed increasing proportions of frozen adult brine shrimp and dry trout pellet (0.047 mm dia.).

Reduction in the density of $f_0$-generation test specimens, "thinning", was not undertaken after the recommended 60-days' toxicant exposure because of appreciable mortality in all test chambers. Rather, excess fish were removed after 5.5-months' chlordane exposure, just prior to anticipated spawning. Concurrently, five spawning substrates, consisting of halved 10 cm i.d., red-clay channel pipe were placed into each adult chamber. Spawning commenced within 24 hr, indicating that the substrates could have been introduced earlier.

From each spawning, all embryos were counted and one to several groups of 50 eggs incubated to determine hatching success. When there were fewer than 50 embryos per spawn or when embryos were spawned on weekends, they were only counted. Incubation cups consisted of polypropylene pipe, 7 cm long and 5 cm i.d., covered at one end with "Nitex" screen (500 μ openings). The cups were oscillated continuously with a rocker-arm assembly (52). At hatching, the numbers of normal, abnormal (i.e. having vertebral abnormalities or otherwise abnormal morphology or behavior), and dead fry were counted. Each rearing chamber was stocked with 50 fry from concurrent hatches, and the $f_1$-generation progeny reared. After 30 days' growth, fry were captured and photographed using the method of McKim and Benoit (53) for measurement of total length. After 60 days all fry were sacrificed and measured to the nearest millimeter for total length and weighed to the nearest milligram after removal of external moisture with toweling. In the first photographic measurement, 20.3 x 25.4 cm black and white prints were made. Thereafter, slides were made of the negatives and projected onto a screen for measurement of lengths. This was believed to have enhanced measurement accuracy, owing to the larger image, and was less costly.

The chronic toxicity test utilizing fathead minnows was terminated after all adults had completed spawning and all fry had been reared for 60 days. Adults were weighed, measured, sexed, and examined for general condition.

### Bluegill

The partial chronic toxicity test utilizing bluegill was conducted with a method recommended by the EPA (54). Since the experiment was not begun with embryos or fry, but with yearlings, the test constituted a partial rather than full chronic because it did not encompass one complete generation. The experimental apparatus consisted of a 2-1 proportional diluter and 12, randomly positioned, 91.4 x 61 x 38 cm tanks containing 178 l of water at a depth of 32 cm. The tanks were illuminated at an average intensity of 1010 lu by two fluorescent lamps (Durotest "Optima" and Sylvania "Gro-Lux"). Photoperiod was regulated to simulate that existing at Evansville, Indiana. The proportional diluter delivered 10 tank volumes per day to each chamber, assuring 90% molecular replacement in about 5 hr.

At the beginning of the test (5 December 1972), juvenile bluegill, obtained from a commercial fish breeder, were anesthetized with ethyl m-aminobenzoate methanesulfonic acid salt (tricaine methanesulfonate), weighed, measured for total length, and 20 specimens randomly distributed into each of the 12 chambers. They were fed twice daily *ad libitum* a dry pelleted ration (Moore-Clark). After 3, 5, and 9.5 months' toxicant exposure, all surviving fish were captured, anesthetized, weighed, and measured for length to determine relative growth.

At 5 months the density of fish in each tank was reduced to three males and seven females in anticipation of spawning. Those which appeared to be sexually immature were discarded.

After 6 months' exposure (6 June 1973) bluegill commenced spawning. The initial spawning occurred 1 month after two 30.5 x 30.5 cm gravel-cement spawning substrates had been placed into each of the 12 chambers. Each substrate had a 24-cm oval depression 4 cm in depth. Generally, fish spawned in the depression, but eggs also tended to be deposited in adjacent areas of the tank, due in part to turbulence produced by the spawning activity. All substrates were checked daily for spawns. Embryos were brushed from the substrate and a number taken for determination of percentage hatch and for the growth and survival studies. The remainder were preserved in 5% formalin for later estimation of spawn size. From each spawn, groups of 100 embryos were incubated in a manner similar to that for fathead minnows, except that 10-l glass rearing chambers (30.5 x 30.5 x 15 cm), which were situated downstream from the 178-l tanks and received test water directly from them (six tank turnovers per day), were used for incubating and rearing the progeny. Substantial embryo mortality consistently occurred as a result of the fungus, Saprolegnia sp., spreading from dead to contiguous living eggs. Although efforts were directed toward obtaining only live embryos for incubation, the substantial amounts of organic debris associated with the adhesive embryos made it difficult to obtain only live embryos or separate dead embryos from within masses of living ones. Treatment of the embryos with 4 mg/l zinc-free malachite green, the level prescribed by Smith (55) for treating flagfish (Jordanella floridae) embryos and found by us to be of some value for treating fathead minnows, was abandoned after initial 5 min baths caused substantial mortality.

Upon hatching, the proportions of normal and abnormal fry were determined and all normal fry reared. Abnormal fry were segregated visually on the basis of physical (e.g. vertebral) defects and erratic behavior. Fry were initially fed blended, cooked chicken egg yolk and "green" water (composed of unicellular chlorophyte algae, protozoans, and copepods) until they became large enough to consume frozen brine shrimp nauplii and "Tetra-Min" tablets crushed to a fine powder. Fry larger than approximately 15 mm in length were offered frozen adult brine shrimp. Acceptance of the dry diet may have been limited. At 30 and 60 days, fry were transferred into a 30.5 x 30.5 cm glass chamber filled with 1.3 cm water and photographed (53). Total lengths of the fry were determined with reference to a metered grid from black and white negatives projected from slides. After 90 days, surviving fry were measured manually for total length and for wet body weight. After the adults had completed spawning and the fry reared for 90 days, the chronic toxicity test was terminated. Adults were weighed, measured for total length, sexed, and examined for general condition.

### Brook Trout

The partial chronic toxicity test using yearling brook trout was begun on 29 March 1973 using procedures and conditions recommended by the U.S. Environmental Protection Agency (56). Just prior to their introduction, the brook trout were anesthetized, measured for total length and weighed. Twelve fish were placed randomly into each of the randomly positioned chambers.

The toxicity testing apparatus consisted of a 4-l proportional diluter and twelve 91.4 x 61 x 38.1 cm glass chambers containing 178 l of water at a depth of 32 cm. The diluter delivered approximately nine tank volumes per day (1602 l), assuring 90% molecular replacement in 5.5 hr. Technical chlordane concentrations were successively diluted by a factor of 0.5. Each tank was covered with screening to retain the fish, and pairs of tanks were illuminated with fluorescent lamps (Durotest "Optima" and Sylvania "Gro-Lux") at an intensity of 1010 lu. The photoperiod simulated that existing at Evansville, Indiana, and the temperature followed the cycle recommended by EPA (56). When the numbers of trout in each tank were reduced in anticipation of spawning, black plastic was used to cover the sides and tops of the tanks in an effort to minimize antagonistic behavior between males in adjacent tanks and provide a more secluded environment for spawning.

Shortly after the test was begun, antagonistic behavior between the fish was observed. Hierarchies were eventually established, but not without considerable fighting. Consequently, up to two fish in each tank developed *Saprolegnia* sp. infestations. Initially, all tanks were treated for 1 min with 67 mg/l zinc-free malachite green and the fungoused areas of the fish painted with a 200 mg/l solution of the compound (57). Both methods were unsuccessful, and the fish perished. After testing different concentrations of malachite green, a level of 0.2 mg/l, employed as a "flush" treatment, was found to be successful in preventing outbreaks of the fungus, but not in arresting an advanced infestation. These treatments were employed once daily for 2 weeks after the fish were handled (i.e. after measurement of growth at 3, 6.5, and 12 months) and during spawning. Several fish also developed what appeared to be bacterial hemorrhagic septicemia subsequent to their initial introduction and after assessment of growth at 3 months. This was successfully treated by incorporating oxytetracycline ("TM-50", Pfizer) into the feed to give a concentration of 0.44% active ingredient or 75 mg A.I./kg/fish/day. Use of oxytetracycline at this level cured some fish and prevented further outbreaks of the disease.

During the test, brook trout were fed a dry pelleted ration (Moore-Clark) at a rate of 2% of their body weight per day. At 3, 6.5 and 12 months, the trout were measured for length and weighed to determine relative growth. After 6.5 months, the numbers of fish residing in each tank were reduced to three males and four females in anticipation of spawning. Since only two of the fish held in 5.8 μg/l chlordane remained, excess control specimens were transferred to one of the 5.8 μg/l tanks. Upon reduction in specimen density, two glass spawning chambers, 30.5 x 25.4 x 10.2 cm, were placed into each tank. The chambers, described by Benoit (58), were designed to simulate a redd.

Brook trout began spawning on 28 December 1974, 8 months after introduction and 1.5 months after "thinning". Each day the number of spawns and embryos per spawn were recorded, and embryos from selected spawns placed into an incubation apparatus (52) for determination of hatching success or viability (development of a neural keel after 12 days). Black plastic was used to shield the developing embryos from light. Viability determinations were usually made on every spawn totaling 20 embryos or more. Determinations of hatching success, which utilized 50 embryos collected from a single spawning, were spread essentially equally throughout the spawning period. Up to eight such determinations were made from the spawns from each of the 12 adult tanks (16 determinations per treatment). Data collected in the hatch study included times to hatching of 50% of the alevins, percentages of normal, abnormal and dead alevins, and total lengths and weights of the alevins. Lengths and weights were determined only for the alevins discarded at hatching.

To assess the effects of chlordane on growth and survival of the progeny, groups of 25 alevins each were reared for 90 days in each of the treatments. Up to four groups of alevins were ultimately used per concentration. These studies were conducted in 37.5 x 18 x 13 cm glass chambers (10 cm depth) which were separated from the adult tanks, received water directly from the diluter, and were covered with screening to retain the fish.

Fry were fed Oregon Moist Pellet trout starter (Moore-Clark) until they were old enough to consume adult brine shrimp and dry pellets. After 30 days' growth, fry were measured for total length using the photographic method (53). At 90 days, fry were measured, weighed and killed.

When the adults from all chambers had not spawned for 2 weeks, they were killed, weighed, measured for total length, and examined for general condition.

## Hyallela azteca

The chronic toxicity test utilizing H. azteca was conducted according to a procedure of EPA (59) and a system consisting of a 2-l proportional diluter and twelve 17.5 x 20.5 x 25 cm glass tanks. Each tank contained 8.3 l of test solution at a depth of 23 cm and was immersed in a water bath to minimize fluctuations in water temperature. The diluter replaced four tank volumes (i.e., 33 l) in 24 hr, a rate equivalent to 90% molecular replacement in approximately 15 hr.

On 26 March 1974, 25 newly hatched H. azteca were introduced into each of the twelve chambers comprising five chlordane concentrations and a control in duplicate. The photoperiod was held at a constant 16-hr light and the water temperature at 17°C. Aspen leaves (Populus sp.), soaked in water for 30 to 60 days prior to feeding, and live Myriophyllum sp. were introduced as food and habitat. Small (2 mm) pellets of fish food (Moore-Clark) were introduced periodically to supplement their diet. The value of the plant and fish pellet as food for this amphipod species was unknown, but both were included because specimens in stock cultures appeared to consume them. The aspen leaves were definitely of dietary importance.

Amphipods were reared under the above conditions for the 65 days of toxicant exposure. Due to time constraints, the test could not be extended through reproduction, although copulating pairs and ovigerous females were observed at termination. At the end of the test, the contents of the test chambers were successively passed through 8, 24, and 100 mesh stainless steel screens (W.S. Tyler Co.) to isolate the amphipods. They were then placed on tissue paper to remove excess moisture and individually weighed on an analytical balance. All specimens from each chamber were then dried to constant weight at 50°C, and the dried specimens analyzed for chlordane residues.

### Daphnia Magna

The chronic toxicity test utilizing *Daphnia magna* was conducted according to a procedure recommended by EPA (60). A 500-ml proportional diluter delivered technical chlordane to a duplicated series of five concentrations and a control. Each glass test chamber measured 28 x 13.5 x 15 cm and contained 5.7 l of test solution. The diluter delivered 3 l to each tank daily, equivalent to replacement of 0.5 tank volumes per day or 90% molecular replacement in approximately 100 hr. Higher flows are believed deleterious to this species (personal communication, K.E. Biesinger, EPA, ERL-D). The diluent was tap water that had been aged at least 2 days in sunlight. Aged rather than ambient tap water was instituted to insure removal of any residual chlorine, which is known to produce effects on *D. magna* at levels as low as 3 µg/l (61).

The 4-week test was begun by introducing 10 first instars, obtained from adults reared in a continuous-flow system, into each of the 12 chambers. The photoperiod was a constant 16-hr light and the water temperature approximately 21°C. The cladocerans were fed daily the blended and sieved mixture of dried yeast, pelleted fish food, and dried alfalfa grass described earlier. Numbers of surviving $f_o$-generation daphnids and $f_1$-generation progeny were counted weekly and the progeny removed. At termination, the progeny produced in the fourth week were removed, composited by treatment, dried to constant weight at 50°C, weighed on an analytical balance, and analyzed for chlordane content.

### Chironomus No. 51

The conduct of the chronic toxicity test of the midge, *Chironomus* No. 51, was similar to that recommended for *C. plumosus* (62). Chlordane was mixed and apportioned in a 2-l proportional diluter operating at a rate sufficient to replace 11.4 tank volumes per day (90% molecular replacement in approximately 5 hr) in 38 x 13 x 18 cm glass chambers containing 4.2 l of water. Each container was covered with screen to contain the adults upon their emergence. The toxicity test was conducted at a water temperature of 25°C and a photoperiod of 16 hr light. The dimming device to simulate dawn and dusk and induce copulation was not used for several reasons. First, it was believed that evaluation of toxicant effects on reproduction had a low probability of success because the helical arrangement of the embryos in the skeins prevented their segregation and accurate enumeration. Secondly, absence of dawn and dusk periods was intended to temporarily delay oviposition, which was known to occur regardless of the occurrence of copulation, and allow harvest of the gravid adults. The latter was apparently successful since only one to two spawnings were observed during the course of testing. At the beginning of the test, newly hatched larvae were randomly distributed into each of the test chambers. They were fed "TetraMin" flakes twice weekly. Adults were captured, sexed, and composited for total dry weight measurement. After determination of dry weight by drying to contant weight at 50°C, the adults were analyzed for chlordane residues.

### Water Quality Analysis

In each chronic toxicity test, six water quality variables, namely dissolved oxygen concentration, pH, total alkalinity, total hardness, and specific conductance, were monitored in the test chambers every week. Replicates were measured on alternate weeks. Water temperatures were continuously recorded and a representative reading taken daily. Measurement of acidity was instituted toward the middle of the project. In the first chronic toxicity tests (fathead minnow and bluegill), measurements were made in each of the six tanks comprising a replicate. Since variation in water quality between treatments was small and was not altered by the presence of chlordane, the carrier, or Triton X-100, water quality analyses in the trout, cladoceran, amphipod, and chironomid tests were confined to the control, mid-range, and high concentrations. Methods of analysis were the same as those described for the acute toxicity tests.

## ANALYSIS OF TECHNICAL CHLORDANE

Stock solutions of technical chlordane were prepared by dissolving the insecticide in double distilled, pesticide-free acetone containing a small amount of the nonionic surfactant, Triton X-100 (Rohm and Haas). The surfactant was intended to enhance the rate of chlordane dissolution in the proportional diluter and decrease the difference between desired and measured insecticide concentrations. The expected concentrations of Triton X-100 were half the nominal level of technical chlordane for both acute and chronic experiments. Thus, for example, in the chronic tests with the three fish species, where desired chlordane concentrations were 0.625, 1.25, 2.5, 5.0 and 10.0 μg/l, the nominal concentrations of the surfactant were 0.3, 0.6, 1.3, 2.5 and 5.0 μg/l, respectively. Although solvent controls were not employed in the chronic tests, the acute and chronic effects of Triton X-100 on brook trout and fathead minnow have been examined in a separate program (63), and concentrations having no chronic effect were uniformly above 100 μg/l.

All water and invertebrate tissue samples were analyzed for technical chlordane with a gas chromatograph equipped with a $^{63}$Ni electron capture detector (Model 990, Perkin-Elmer Corp.). The 183 cm long, 0.64 cm o.d. glass column was packed with 2% SE-30 on 100 - 120 mesh "Gas Chrom Q". The carrier gas was nitrogen, and the flow rate was 60 ml/min. Oven temperatures varied depending upon the type of sample being analyzed.

For measurement of technical chlordane in water, 10.0 to 20.0 ml water samples were extracted once for 1 min with 2.0 to 5.0 ml portions of pesticide-grade n-hexane. The larger sample volumes were applied to determinations made in the chronic toxicity tests, whereas smaller sample volumes sufficed for measurements made in acute toxicity tests. One to 10 microliters of sample extract were injected into the chromatograph. The chromatograms were measured for peak area with a planimeter and compared to technical chlordane standards. Separation of the chlordane constituents was performed with isothermal conditions (200°C) and detector, manifold, and injector temperatures of 240°C.

The accuracy and reproducibility of the method were checked by spiking technical chlordane into laboratory water. Samples containing technical chlordane concentrations of 10 µg/l were analyzed with a recovery of 100.2 ± 5.2%, while concentrations as low as 0.5 µg/l were recovered to the extent of 87.2 ± 11.0%. The coefficients of variation for the 0.5 and 10.0 µg/l concentrations were 12.6 and 5.2%, respectively.

Analyses of the contents of the various technical chlordane constituents in the three fish species were considered unreliable due to several technical errors which were not identified and corrected in time to have the analyses repeated. These problems were corrected prior to analyses of technical chlordane residues in the three invertebrate species.

Whole body extracts of dried samples of the invertebrates were obtained by grinding the specimens in a tissue grinder with pesticide residue-free petroleum ether (30° to 75°C). One to 10 microliter volumes of the extract, which was used without further manipulation, were separated on the gas chromatograph using a 160° to 240°C temperature program (6°C/min) and the operating conditions described for chlordane analysis in water. No major interferences were detected in the chromatograms; heptachlor, the chlordenes, cis-chlordane, trans-chlordane, cis-nonachlor, and trans-nonachlor were selected for quantitation.

## STATISTICAL ANALYSIS

### Acute Toxicity Tests

Concentration-percent mortality data were analyzed with logarithmic-probability (log-probit) methods using either the manual procedure of Litchfield and Wilcoxon (64) or the computer program of Dixon (65). Computer processing was accomplished using IBM 360/75 hardware. The log-probit method was selected because it is a more objective approach than the graphical interpolation method, offers a test of the regression line's goodness-of-fit, and provides the statistics necessary for calculating 95% confidence limits for median lethal concentrations (LC50) and for comparing differences between two LC50 values.

For homogeneous data, upper and lower 95% confidence limits for LC50 value were calculated as (LC50)(f) and LC50/f, respectively, where f is the antilogarithm of $1.96\ \theta\ (N'/2)^{-1/2}$, $\theta$ is the standard deviation of the logarithm of the population tolerance frequency distribution, and N' is the number of test animals expected to have perished within the percent mortality interval of 16 to 84% (64). An equivalent equation is ± 1.96 times the standard error of the log LC50 (66). For heterogeneous data, i.e. where chi-square analysis of the fitted line indicated lack of goodness-of-fit, the following equation was used: $f = (\text{Student's t-value}) (\sigma) (\chi^2/n)^{1/2} (N'/2^{-1/2}$, where n is the number of concentrations used in calculating the LC50 (66). The logarithms of the median lethal concentrations were plotted against the logarithms of the exposure times to give toxicity curves (45) for the fish species and D. magna.

The accuracy of the LC50 estimates and their 95% confidence limits, generated by the Litchfield and Wilcoxon (64) and computer program (65) methods, were compared with similar calculations made by eight other aquatic toxicology laboratories using standard concentration-percent response data (Appendix Table 3) supplied by The Committee on Methods for Toxicity Tests with Aquatic Organisms. Our LC50 estimates were in agreement with the average LC50 computed by the other laboratories (Appendix Table 4) using both methods of analysis, but the 95% confidence limits were usually narrower with the computer method than with that of Litchfield and Wilcoxon (64).

Median lethal times (LT50) for measured toxicant concentrations were calculated in some cases. The LT50 is the time required for 50% of the test specimens to die in a given concentration. Data were analyzed in the same manner as for the calculation of LC50 values and were plotted on the same toxicity curve, with the exception that 95% confidence limits were determined for the independent variable, time, rather than the LC50.

Control mortality occurred in less than 5% of the toxicity tests and was less than 10% in all cases. Median response estimates were corrected for any control mortality with the computer program method.

Several statistical comparisons were made to determine the significance of differences between the 96-hr LC50 values of the different species using data generated by probit analysis. The equation, Student's $t = (\hat{\mu}_1 = \hat{\mu}_2) (\theta_1^2/N'_1 /2 + \theta_2^2/N'_2 /2)^{-1/2}$, was used to test significance. The symbol $\hat{\mu}$ denotes an LC50 and subscripts pertain to the particular LC50 values being compared. The degrees of freedom were $n_1 + n_2 - 2$, where n is the total number of test specimens employed to derive an LC50 estimate.

## Chronic Toxicity Tests

Analysis of variance with a one-way design was used exclusively in evaluating the significance of differences between treatments. Dunnett's test (67) was used to determine whether controls were different from each of the other treatments. Log-probit analysis was used to determine median emergence times of adult chironomids in the chronic tests of that species.

# SECTION VI

# RESULTS

## ACUTE TOXICITY TESTS

### Water Quality and Toxicant Concentrations

Water quality during the various acute toxicity tests is summarized in Table 4. Water temperatures were 25°C in tests of fathead minnow and bluegill, 15°C in the tests of brook trout and *H. azteca*, and 21°C in that of *D. magna*. Dissolved oxygen concentrations were greater than 70% in all toxicity tests except the second one employing brook trout where it was 62% of air saturation. The diluent water was moderately alkaline and of intermediate hardness.

Concentrations of technical chlordane were determined from two to five times during each test, depending upon exposure time, and are summarized in Table 5.

### Toxicity

The order of decreasing species sensitivity to acutely lethal concentrations of technical chlordane was *D. magna*, fathead minnow, brook trout, bluegill, and *H. azteca*. Median effective concentrations ranged from 96-hr values of 28.4 and 35.2 μg/l for *D. magna* to a 168-hr value of 97.1 μg/l for *H. azteca*. Too few *H. azteca* had perished at 96 hr to permit an EC50 estimate for this period of exposure (Appendix Table 5). The 96-hr LC50's were 36.9 μg/l for fathead minnow, 45 μg/l for brook trout, and 59 μg/l for bluegill (Appendix Tables 6, 7, and 8).

Comparisons were made to determine the significance of differences between species in terms of their respective 96-hr EC50 and LC50 values. As shown in Appendix Table 9, the only statistically significant differences were between *D. magna* and bluegill and brook trout ($p < 0.001$). None of the fish species were significantly different in sensitivity.

Chlordane exposures were sufficiently long (up to 192 hr) in the acute toxicity tests to allow partial delineation of toxicity curves (Fig. 2). Linear curves described the toxicity of chlordane to brook trout, bluegill, and possibly to *D. magna*, whereas a rectangular hyperbola characterized its toxicity to fathead minnows. The 192-hr LC50 of 32 μg/l may be considered an estimate of the median lethal threshold of technical chlordane for fathead minnow--the concentration at which acute lethality to 50% of the test specimens ceases.

TABLE 4. WATER QUALITY DURING ACUTE TOXICITY TESTS OF TECHNICAL CHLORDANE

| Species | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l CaCO$_3$ | Acidity, mg/l CaCO$_3$ | Total hardness, mg/l CaCO$_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| Fathead minnow | 24.8[a]<br>±0.4<br>(23) | 6.5<br>±0.3<br>(33) | 77.1<br>±3.3<br>(33) | 7.70<br>±0.14<br>(28) | 169<br>±1<br>(28) | ...[b] | 152<br>±1<br>(28) | 370[c]<br>±24<br>(5) |
| Bluegill | 25.3<br>±0.2<br>(24) | 6.4<br>±0.3<br>(14) | 76.5<br>±3.7<br>(14) | 7.63<br>±0.10<br>(12) | 160<br>±2<br>(11) | ... | 161<br>±1<br>(11) | 393<br>±4<br>(2) |
| Brook trout | | | | | | | | |
| Test 1 | 14.7<br>±0.6<br>(24) | 7.5<br>±1.5<br>(9) | 73.2<br>±14.9<br>(9) | 7.71<br>±0.41<br>(9) | 155<br>±9<br>(9) | 11.6<br>±2.8<br>(9) | 149<br>±13<br>(9) | 393<br>±26<br>(3) |
| Test 2 | 15.2<br>±0.5<br>(15) | 6.6<br>±1.5<br>(10) | 61.7<br>±16.8<br>(10) | 7.70<br>±0.14<br>(10) | 155<br>±4<br>(10) | 6.5<br>±1.8<br>(10) | 135<br>±2<br>(10) | 362<br>±4<br>(3) |
| *Daphnia magna* | | | | | | | | |
| Test 1 | 20.9<br>±0.3<br>(6) | 6.8<br>±0.1<br>(6) | 78.8<br>±2.3<br>(6) | 8.07<br>±0.04<br>(10) | 144<br>±2<br>(10) | 6.7<br>±1.7<br>(10) | 143<br>±3<br>(10) | 372<br>±0<br>(3) |

Continued ....

TABLE 4. WATER QUALITY DURING ACUTE TOXICITY TESTS OF TECHNICAL CHLORDANE--continued

| Species | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Acidity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| Daphnia magna | | | | | | | | |
| Test 2 | 20.8<br>±0.5<br>(9) | 6.6<br>±0.6<br>(6) | 72.9<br>±6.6<br>(6) | 8.04<br>±0.09<br>(6) | 149<br>±3<br>(6) | 5.4<br>±2.2<br>(6) | 154<br>±8<br>(6) | 394<br>±9<br>(2) |
| Hyallela azteca | 15.5<br>±0.5<br>(8) | 7.2<br>±0.3<br>(7) | 71.4<br>±3.3<br>(7) | 7.85<br>±0.18<br>(13) | 156<br>±4<br>(13) | 6.0<br>±3.0<br>(13) | 144<br>±2<br>(13) | 363<br>±7<br>(4) |

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[a] Means ±1 standard deviation, and number of measurements made per parameter.

[b] No observation.

[c] Specific conductance measurements were composites taken at each sampling time.

## TABLE 5. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN ACUTE TOXICITY TESTS

| Test species | No measurements per test | Chlordane concentration, μg/l | | | | | |
|---|---|---|---|---|---|---|---|
| | | Tank I | Tank II | Tank III | Tank IV | Tank V | Tank VI |
| Fathead minnow | 3 | N.D.[a] | 12.3<br>±1.1 | 20.0<br>±6.5 | 28.4<br>±14.5 | 34.1<br>±9.4 | 53.4<br>±10.0 |
| Bluegill | 4 | 0.06<br>±0.3 | 12.8<br>±3.1 | 39.2<br>±7.2 | 59.1<br>±3.4 | 81.5<br>±0.1 | 104.3<br>±9.8 |
| Brook trout | | | | | | | |
| Test 1 | 5 | N.D. | 12.4<br>±3.6 | 17.8<br>±10.2 | 20.0<br>±7.2 | 34.2<br>±8.2 | 41.0<br>±15.2 |
| Test 2 | 2 | N.D. | 21.0<br>±0.6 | 37.2<br>±3.5 | 52.9<br>±34.8 | 117<br>±63 | 125<br>±104 |
| *Daphnia magna* | | | | | | | |
| Test 1 | 3 | N.D. | 10.4<br>±5.0 | 16.5<br>±2.4 | 21.8<br>±2.6 | 28.4<br>±6.2 | 33.9<br>±3.7 |
| Test 2 | 3 | N.D. | 10.4<br>±4.2 | 14.4<br>±5.2 | 20.8<br>±7.6 | 28.3<br>±11.5 | 42.8<br>±16.1 |
| *Hyallela azteca* | 4 | N.D. | 35.3<br>±12.3 | 66.7<br>±10.3 | 83.7<br>±11.9 | 115.2<br>±13.5 | 161.3<br>±15.3 |

[a] No technical chlordane detected.

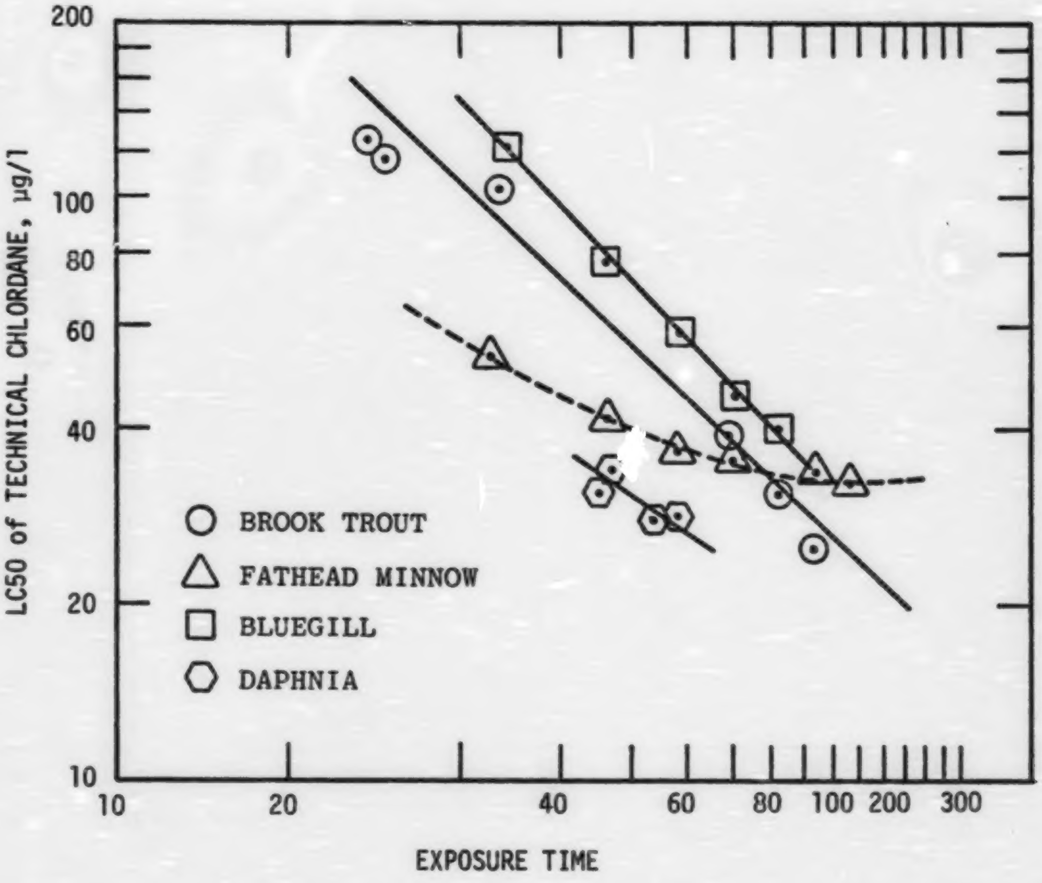

Fig. 2. Toxicity curves for three fish species and Daphnia magna exposed to technical chlordane.

## CHRONIC TOXICITY TO FATHEAD MINNOWS

### Water Quality and Chlordane Concentrations

Water quality during the chronic toxicity test is summarized in Appendix Table 10. Since the concentrations of chlordane, acetone, and Triton X-100 had no apparent influence on the water quality parameters measured, the data for each week were pooled and mean values reported. Water temperatures were approximately 21°C upon initiation of toxicant exposure, but were raised to 25°C during a 6-week interval and maintained within a degree of that temperature for the duration of the experiment. Problems with the temperature control apparatus prevented starting the test at 25°C. Dissolved oxygen concentrations were maintained at greater than 60% of air saturation without artificial aeration. Alkalinity, pH, and hardness levels of the ambient water were very uniform.

Concentrations of technical chlordane, measured in the six tanks comprising each replicate, are summarized in Appendix Table 11. In general, measured concentrations were 55% of desired. Although the reasons for the loss were not ascertained, they could have been due to chlordane adsorption to the surfaces of the test apparatus, to organic material, or because of assimilation by the test organisms and epiphytes. Chlordane analysis of hexane extracts of scrapings of algal-bacterial mats from the walls of the tubes running between the diluter and the test chambers indicated that substantial amounts of the pesticide were associated with these organisms. Measured aqueous concentrations of technical chlordane averaged 0.36 ± 0.16, 0.75 ± 0.25, 1.38 ± 0.57, 2.78 ± 1.06, and 6.03 ± 2.25 µg/l. Traces of chlordane were sometimes detected in control chambers.

### Chronic Effects of Chlordane on Survival, Growth, and Reproduction

Under the aforementioned experimental conditions, fathead minnows were cultured through one generation. Growth and survival of fathead minnows introduced as 1- to 5-day-old fry (termed $f_o$-generation) was very poor, apparently because the food ("TetraMin") was not small enough for all the fry and possibly not of adequate nutritive value. Regardless of the quality of the diet, chlordane may have slightly retarded growth of fry exposed for the first 30 days to concentrations at and above 2.78 µg/l (Table 6). Analysis of variance of the growth data did not indicate that there were any statistically significant differences between the controls and the treatment groups at the 95% level of confidence. After 60 days, there were no apparent differences in growth, leading to the conclusion that the insecticide had no significant adverse effect at the concentrations employed.

At the end of the chronic test, minnows reared in 6.03 µg/l chlordane were significantly larger ($p < 0.01$) than controls (Table 7). However, the importance of this difference is suspect because only eight individuals remained in the high concentration at termination relative to 20 to 30 fish in each of the other treatments.

## TABLE 6. TOTAL LENGTHS OF FATHEAD MINNOW FRY CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Total length, mm | | | |
|---|---|---|---|---|
| | 30 days | | 60 days | |
| | $F_0$-gen.[a] | $F_1$-gen.[b] | $F_0$-gen. | $F_1$-gen. |
| Control | 9.7[c]<br>±2.6 | 13.3<br>±2.4 | 12.3<br>±2.8 | 21.4<br>±4.5 |
| Control | 8.4<br>±1.7 | 12.1<br>±1.9 | 10.5<br>±2.8 | 17.7<br>±3.2 |
| 0.36 | 8.0<br>±1.2 | 11.4<br>±1.8 | 12.9<br>±2.7 | 19.1<br>±4.3 |
| 0.36 | 8.8<br>±2.1 | 12.5<br>±2.4 | 12.8<br>±4.3 | 19.4<br>±4.4 |
| 0.75 | 8.4<br>±1.4 | 11.1<br>±1.9 | 12.2<br>±5.2 | 20.3<br>±3.2 |
| 0.75 | 8.5<br>±0.8 | 12.7<br>±2.4 | 12.5<br>±3.7 | 18.7<br>±3.9 |
| 1.38 | 8.3<br>±2.1 | 8.6<br>±1.5 | 12.0<br>±4.2 | 18.2<br>±3.7 |
| 1.38 | 8.3<br>±2.2 | 10.3<br>±1.7 | 12.0<br>±3.6 | 20.5<br>±4.2 |
| 2.78 | 7.4<br>±1.2 | 12.0<br>±2.1 | 10.7<br>±3.3 | 19.1<br>±5.0 |
| 2.78 | 7.6<br>±1.1 | 11.8<br>±2.6 | 12.2<br>±4.7 | 19.3<br>±4.6 |
| 6.03 | 6.9<br>±0.9 | 11.3<br>±2.6 | 9.5<br>±2.0 | 19.8<br>±5.5 |
| 6.03 | 7.4<br>±1.5 | 11.4<br>±1.7 | 12.5<br>±3.4 | 21.4<br>±4.4 |

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[a] $F_0$-generation constitutes 1- to 5-day-old fry from parents having no known previous history of chlordane exposure.

[b] $F_1$-generation represents progeny spawned by adults having 6 months' chlordane exposure.

[c] Mean ±1 standard deviation.

### TABLE 7. LENGTHS AND WEIGHTS OF ADULT FATHEAD MINNOWS AT TERMINATION OF CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

| Meas. chlordane conc., μg/l | No. fish | Total length, mm | Wet body weight, g |
|---|---|---|---|
| Control | 14 | 46[a]<br>±5 | 1.2<br>±0.5 |
| Control | 15 | 49<br>±5 | 1.5<br>±0.7 |
| 0.36 | 15 | 47<br>±5 | 1.0<br>±0.6 |
| 0.36 | 15 | 53<br>±5 | 1.7<br>±0.6 |
| 0.75 | 13 | 50<br>±8 | 1.3<br>±0.7 |
| 0.75 | 13 | 53<br>±6 | 1.4<br>±0.7 |
| 1.38 | 15 | 48<br>±6 | 1.1<br>±0.5 |
| 1.38 | 17 | 48<br>±7 | 1.3<br>±0.7 |
| 2.78 | 8 | 54<br>±8 | 1.7<br>±0.7 |
| 2.78 | 13 | 50<br>±6 | 1.3<br>±0.7 |
| 6.03 | 3 | 59<br>±6 | 1.9<br>±0.9 |
| 6.03 | 5 | 56<br>±4 | 1.7<br>±0.4 |

[a] Mean ±1 standard deviation. With the exception of one of the controls, fish which died during spawning are included in the summaries.

Mortality of minnows was extensive during the first month, particularly in the first 2 weeks, and was presumed to be due to an inadequate diet. Although this was later corrected with a better diet, the poor early survival of the $f_0$-generation fry tended to obscure the relationship between chlordane concentration and survival for the first 8 weeks of the test (Table 8). Subsequent mortality, i.e. from 8 weeks up to the time of spawning at 24 weeks, was negligible, with fewer than three fish dying in any treatment. Mortality during spawning was extensive, particularly in fish exposed to chlordane concentrations greater than 0.75 μg/l. Although variation in mortality between treatments was considerable, it appears that chlordane posed an additional stress on the fish at a time when they were naturally stressed.

Details of spawning activity, embryo production, and hatching of fry are given in Table 9. In general chlordane had no effect on the number of spawnings, embryos produced per female, or the percentages of normal, abnormal, or dead fry observed at hatching. Problems were regularly encountered with fungus on incubating embryos. In the majority of cases, *S. parasitica* on dead embryos spread to adjacent living ones despite daily immersion for 5 min in a 4 mg/l solution of zinc-free malachite green. This problem occurred in all treatments including controls.

Subsequent growth of the $f_1$-generation fry is tabulated in Tables 6 and 10. As also observed for $f_0$-generation fry, the second generation progeny were slighly smaller after 30 days in 6.03 μg/l chlordane than the controls, although differences were not statistically significant. After 60 days, there were no significant differences in size between fish in the five chlordane concentrations and the control.

Mean mortality of the $f_1$-generation through the first 60 days ranged from 15 to 40% and was not increased by any of the chlordane concentrations used (Table 10).

Statistical analysis of the results indicated that none of the concentrations employed had any significant deleterious effects on any of the life cycle stages of the fathead minnow. On the other hand, one apparent effect from several of the chlordane concentrations was suggested, in that concentrations greater than 0.75 μg/l caused increased mortality of adult minnows during the period of spawning. Although this result is conceivable since adults are already stressed and incur mortality naturally during spawning, its significance can be challenged because of the high fry mortality that occurred in all treatments during the first 2 months of the experiment. Without repeating the test, the question of whether these fish were already in a weakened condition will remain.

TABLE 8. MORTALITY OF $F_0$-GENERATION FATHEAD MINNOWS DURING CHRONIC EXPOSURE TO CHLORDANE

| Meas. chlordane conc., μg/l | Cumulative percent mortality[a] | | | | % mortality during spawning[c] |
|---|---|---|---|---|---|
| | 4 weeks | 8 weeks | 12 weeks | 24 weeks[b] | |
| Control | 82 | 86 | 86 | 86 | 6.6 |
| Control | 80 | 82 | 82 | 82 | 0 |
| 0.36 | 72 | 78 | 78 | 78 | 13.3 |
| 0.36 | 44 | 50 | 50 | 52 | 26.7 |
| 0.75 | 56 | 70 | 72 | 72 | 23.1 |
| 0.75 | 70 | 72 | 72 | 72 | 0 |
| 1.38 | 50 | 64 | 68 | 68 | 40.0 |
| 1.38 | 56 | 64 | 64 | 66 | 93.3 |
| 2.78 | 70 | 80 | 80 | 82 | 75.0 |
| 2.78 | 68 | 70 | 74 | 76 | 15.4 |
| 6.03 | 78 | 94 | 94 | 94 | 33.3 |
| 6.03 | 66 | 90 | 90 | 90 | 20.0 |

[a] Based on a 50 fish per concentration.

[b] Fish "thinned" at this time, which was just prior to the commencement of spawning. Excess males were removed and minnows of equivalent age from the culture facility were used to bring the densities in the control tanks to 15 fish each.

[c] Based on 15 fish in each of the control and 0.36 μg/l concentrations, and 26 fish (13 and 13) in 0.75 μg/l, 32 fish (15 and 17) in 1.38 μg/l, 21 fish (8 and 13) in 2.78 μg/l, and 8 fish (3 and 5) in 6.03 μg/l chlordane at the beginning of spawning.

TABLE 9. SPAWNING HISTORY OF FATHEAD MINNOWS CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| | Measured concentration of chlordane, µg/l | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Control | Control | 0.36 | 0.36 | 0.75 | 0.75 | 1.38 | 1.38 | 2.78[a] | 2.78 | 6.03 | 6.03 |
| No. females | 9 | 7 | 11 | 6 | 8 | 8 | 12 | 7 | 3 | 7 | 1 | 2 |
| No. of spawnings | 30 | 16 | 25 | 12 | 16 | 10 | 23 | 14 | 2 | 19 | 6 | 10 |
| Avg. No. spawnings/ female | 3.33 | 2.29 | 2.27 | 2.00 | 2.00 | 1.25 | 1.92 | 2.00 | 0.67 | 2.71 | 6.00 | 5.00 |
| No. eggs | 4,960 | 1,262 | 3,788 | 627 | 1,949 | 487 | 2,970 | 1,550 | 20 | 1,926 | 527 | 576 |
| Avg. No. eggs/ female | 551 | 180 | 344 | 105 | 244 | 61 | 248 | 221 | 20 | 275 | 527 | 288 |
| Avg. No. eggs/ spawning | 165 | 79 | 152 | 52 | 122 | 49 | 129 | 111 | 10 | 101 | 88 | 58 |
| Percent hatch | 37.5 | 27.3 | 60.1 | 29.1 | 72.5 | 38.2 | 55.7 | 40.0 | 2.8 | 46.0 | 44.5 | 54.9 |
| Percent abnormal fry | 1.7 | 0.8 | 3.6 | 1.4 | 2.0 | 0.2 | 2.3 | 1.2 | 0 | 1.2 | 0.9 | 2.9 |
| Percent dead fry | 3.7 | 1.8 | 4.6 | 6.1 | 12.2 | 0.1 | 3.7 | 4.9 | 0 | 6.7 | 3.0 | 5.2 |

[a] Due to complete mortality of mature males during spawning, males and females co-existed for only the first 3 weeks of spawning.

### TABLE 10. MORTALITY AND RELATIVE SIZE OF $F_1$-GENERATION FATHEAD MINNOWS CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | After 60 days' exposure | |
|---|---|---|
| | Mortality, % | Wet body weight, g |
| Control | 40[a]<br>±14<br>(3) | 0.113[b]<br>±0.062<br>(90) |
| 0.36 | 23<br>±19<br>(4) | 0.094<br>±0.071<br>(156) |
| 0.75 | 15<br>±6<br>(3) | 0.096<br>±0.047<br>(128) |
| 1.38 | 38<br>±16<br>(3) | 0.106<br>±0.067<br>(93) |
| 2.78 | 28<br>±17<br>(4) | 0.095<br>±0.064<br>(145) |
| 6.03 | 28<br>±19<br>(4) | 0.117<br>±0.078<br>(144) |

[a] Mean ±1 standard deviation and number of groups of 50 fry tested.

[b] Weighted mean ±1 standard deviation and number of individuals from which estimate made.

## CHRONIC TOXICITY TO BLUEGILL

### Water Quality and Chlordane Concentrations

The results of the water quality monitoring program for the bluegill chronic are summarized in Appendix Table 12. Water temperatures were gradually adjusted from an initial level of 19°C to a temperature of 28°C, which was required for induction of spawning. Thereafter, they were maintained at 28°C for the duration of the experiment. From the inception of testing, on 5 December 1972, until 31 March 1973, no aeration of the test chambers was necessary. But as of the first of April 1973, the percentage saturation of dissolved oxygen had dropped to below the required minimum of 60%, and artificial aeration with oil-free compressed air was instituted. During the experiment, the other variables remained relatively constant.

Concentrations of technical chlordane were measured in each set of six treatments every other week. Since data collected prior to 26 March 1973 were considered unreliable because of analytical problems, they were not included in the summary in Appendix Table 13. Mean measured concentrations ranged from 40 to 52% of desired and averaged 0.25 ± 0.12, 0.54 ± 0.21, 1.22 ± 0.53, 2.20 ± 0.56 and 5.17 ± 1.57 μg/l.

### Chronic Effects on Survival, Growth and Reproduction

At the beginning of the chronic test (5 December 1972), 20 yearling bluegill averaging 144 mm in total length and 58 g in wet body weight were introduced into each of the 12 test chambers. During the 9.5 months of continuous toxicant exposure, they grew an average of 19% in total length, to 172 mm, and 78% in wet body weight, to 103 g (Table 11). Chlordane did not have any statistically significant effect on growth of $f_0$-generation bluegills at either 3, 5, or 9.5 months.

Aside from an anomalous mortality totalling 35% in one of the control replicates, which was tentatively assigned to a brief outbreak of bacterial hemorrhagic septicemia, bluegill mortality was largely confined to the 2.20 and 5.17 μg/l concentrations (Table 12). Bluegill began dying in greater numbers from 12 to 22 weeks in the 5.17 μg/l concentration, but experienced highest mortality during the period of spawning (22 - 37 weeks). Mortality of fish exposed to 2.20 μg/l chlordane was also confined largely to the 15 weeks of spawning activity. None of the fish exposed to toxicant concentrations lower than 2.20 μg/l died. None of those which died between 12 and 22 weeks or during spawning showed evidence of erratic behavior or disease. Moribund fish died passively. Bluegill held in the two highest concentrations had greatly diminished appetites relative to the other groups.

Bluegill began spawning on 8 June 1973, 6 months after the beginning of the chronic test. Embryo production was greatest in the control, 0.25 and 0.54 μg/l concentrations, was substantially reduced in the 1.22 μg/l level, and did not occur in the 2.20 and 5.17 μg/l concentrations (Table 13). Hatching success ranged from 25.0 to 70.5% and did not appear to be

## TABLE 11. GROWTH OF $F_0$-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Total length, mm | | | | Wet body weight, g | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 months | 3 months | 5 months | 9.5 months | 0 months | 3 months | 5 months | 9.5 months |
| Control | 143[a]<br>±17 | 155<br>±22 | 161<br>±19 | 167<br>±19 | 58<br>±27 | 81<br>±33 | 99<br>±31 | 98<br>±38 |
| Control | 143<br>±16 | 158<br>±18 | 163<br>±19 | 172<br>±24 | 58<br>±23 | 81<br>±32 | 101<br>±36 | 104<br>±50 |
| 0.25 | 142<br>±13 | 154<br>±18 | 161<br>±15 | 173<br>±17 | 53<br>±15 | 75<br>±24 | 94<br>±26 | 105<br>±29 |
| 0.25 | 147<br>±19 | 158<br>±21 | 163<br>±17 | 172<br>±19 | 63<br>±27 | 85<br>±32 | 102<br>±33 | 103<br>±38 |
| 0.54 | 143<br>±15 | 150<br>±17 | 157<br>±14 | 174<br>±14 | 58<br>±22 | 70<br>±24 | 87<br>±25 | 107<br>±30 |
| 0.54 | 141<br>±14 | 153<br>±15 | 159<br>±15 | 168<br>±20 | 53<br>±17 | 75<br>±24 | 96<br>±33 | 102<br>±42 |
| 1.22 | 146<br>±21 | 160<br>±23 | 166<br>±20 | 169<br>±27 | 64<br>±28 | 84<br>±34 | 107<br>±39 | 110<br>±58 |

Continued ....

## TABLE 11. GROWTH OF $F_o$-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE--continued

| Meas. chlordane conc., µg/l | Total length, mm | | | | Wet body weight, g | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 months | 3 months | 5 months | 9.5 months | 0 months | 3 months | 5 months | 9.5 months |
| 1.22 | 146<br>±17 | 159<br>±17 | 160<br>±15 | 172<br>±16 | 59<br>±21 | 78<br>±26 | 92<br>±30 | 106<br>±37 |
| 2.20 | 146<br>±18 | 158<br>±21 | 160<br>±16 | 179<br>±13 | 58<br>±13 | 79<br>±22 | 91<br>±28 | 98<br>±20 |
| 2.20 | 145<br>±17 | 156<br>±21 | 162<br>±19 | 168<br>±22 | 58<br>±25 | 78<br>±34 | 100<br>±38 | 97<br>±52 |
| 5.17 | 143<br>±11 | 153<br>±15 | 160<br>±18 | 178<br>± 9 | 57<br>±19 | 69<br>±27 | 95<br>±35 | 106<br>±24 |
| 5.17 | 147<br>±20 | 164<br>±24 | 166<br>±23 | ...[b] | 63<br>±30 | 90<br>±34 | 100<br>±42 | ...[b] |

[a] Mean ±1 standard deviation.

[b] No fish remaining.

## TABLE 12. MORTALITY OF $F_o$-GENERATION BLUEGILL DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Cumulative percent mortality[a] | | | | No. fish remaining after thinning | % mortality during spawning, 22-37 weeks |
|---|---|---|---|---|---|---|
| | 4 weeks | 8 weeks | 12 weeks | 22 weeks | | |
| Control | 10.0 | 20.0 | 35.0 | 35.0 | 8 | 0.0 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0.0 |
| 0.25 | 0.0 | 0.0 | 0.0 | 5.0 | 10 | 0.0 |
| 0.25 | 0.0 | 0.0 | 0.0 | 0.0 | 10 | 0.0 |
| 0.54 | 0.0 | 0.0 | 10.0 | 10.0 | 10 | 0.0 |
| 0.54 | 10.0 | 10.0 | 10.0 | 15.0 | 9 | 0.0 |
| 1.22 | 0.0 | 0.0 | 0.0 | 0.0 | 9 | 0.0 |
| 1.22 | 0.0 | 0.0 | 5.0 | 5.0 | 10 | 0.0 |
| 2.20 | 10.0 | 10.0 | 10.0 | 10.0 | 10 | 0.0 |
| 2.20 | 0.0 | 5.0 | 5.0 | 10.0 | 10 | 30.0 |
| 5.17 | 0.0 | 10.0 | 15.0 | 25.0 | 8 | 12.5 |
| 5.17 | 0.0 | 15.0 | 35.0 | 60.0 | 7 | 100.0 |

[a] Based on 20 fish per chamber. At thinning, numbers of fish were reduced to a maximum of 10 per chamber.

## TABLE 13. SPAWNING HISTORY OF BLUEGILL CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., μg/l | No. females | Mean No. spawnings/ female | Mean No. embryos/ female | Mean No. embryos/ spawn | % hatch | % fry | |
|---|---|---|---|---|---|---|---|
| | | | | | | Abnormal | Dead |
| Control[a] | 5 | 0.20 | 20 | 100 | 65.5 | 1.0 | 0 |
| Control | 7 | 0.43 | 2,252 | 5,255 | 63.8 | 1.7 | 0.8 |
| 0.25 | 6 | 0.33 | 400 | 1,200 | 51.8 | 0.6 | 0.9 |
| 0.27 | 7 | 0.57 | 3,559 | 6,229 | 32.5 | 1.1 | 0 |
| 0.54 | 6 | 0.67 | 3,442 | 1,561 | 25.0 | 0 | 0.2 |
| 0.54 | 6 | 0.50 | 2,074 | 4,148 | 25.1 | 0.6 | 0.3 |
| 1.22 | 6 | 0.17 | 263 | 1,575 | 45.5 | 0.7 | 0.7 |
| 1.22 | 7 | 0 | 0 | 0 | ... | ... | ... |
| 2.20 | 7 | 0 | 0 | 0 | 54.5[b] | 0.5 | 0 |
| 2.20 | 7 | 0 | 0 | 0 | 66.5[b] | 1.0 | 0.5 |
| 5.17 | 7 | 0 | 0 | 0 | 32.5[b] | 7.5 | 7.5 |
| 5.17 | 3 | 0 | 0 | 0 | 70.5[b] | 1.5 | 0.5 |

[a] Testes of male fish appeared to be largely immature.

[b] Control eggs incubated.

influenced by the levels of technical chlordane tested. Embryos spawned by control bluegill and transferred to the 2.20 and 5.17 µg/l concentrations hatched in proportions which overlapped those of embryos spawned by fish in the other treatments. However, there were slightly greater proportions of dead and abnormal fry (i.e. erratic swimming behavior or structural defects) at hatch in the 5.17 µg/l concentration.

When it had been determined that all bluegill had completed spawning, they were killed, weighed, measured for total length, and the condition and size of their gonads assessed and measured (Table 14). Although the majority of both sexes had well developed and essentially unspent gonads, those of males in one of the control replicates appeared to be immature and weighed less than those of males from most of the other treatments. Testes of bluegill exposed to 5.17 µg/l were also generally smaller than those of other male fish. Since these fish did not spawn, the effect of successful spawning in reducing testicular mass was not a factor. In contrast to the differences in gonadal development noted for males, females had well-developed ovaries. The ovaries of fish exposed to 2.20 µg/l were somewhat smaller than other groups and these fish did not spawn. These observations suggest that the test conditions were not conducive to spawning or the fish were not old enough. According to reports by others (D. T. Allison, EPA, ERL-D, personal communication), most of the bluegill may have simply been too young.

Few progeny were available for growth and survival studies, and the results are considered inconclusive. Survival data, detailed in Table 15, did not indicate treatment effects. Growth data suggested that control fish grew less rapidly than those in the intermediate concentrations. Only in the 2.20 µg/l treatment was there a suggestion of diminished growth (Table 16). All of the fry reared in 5.17 µg/l chlordane died within 30 days.

In summary the most consequential effect of technical chlordane on bluegill was inhibition of reproduction at the 2.20 and 5.17 µg/l levels. Apparent but non-significant effects were noted on reproduction in the 1.22 µg/l concentration and on hatching success in the lowest concentration tested, 0.25 µg/l. Bluegill did not spawn in one of the 1.22 µg/l replicates and hatching success of fry in chambers receiving chlordane was no greater than 80% that of controls. Thus, although adverse chronic effects are certainly possible at lower concentrations, the 2.20 µg/l was the lowest level at which definite effects were observed.

## CHRONIC TOXICITY TO BROOK TROUT

### Water Quality and Chlordane Concentrations

During the 13-month duration of the chronic toxicity test utilizing brook trout, there were only small fluctuations in water quality. Dissolved oxygen concentrations averaged at least 60% of air saturation, although artificial aeration was needed to maintain this level after the test had been in progress for 2.5 months (Appendix Table 14).

## TABLE 14. CONDITIONS OF ADULT BLUEGILL AT TERMINATION OF CHRONIC TOXICITY TEST

| Meas. chlordane conc., µg/l | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|
| | Total length, mm | Wet weight, g | Testes weight, mg | Testes weight, mg/g | Total length, mm | Wet weight, g | Ovary weight, mg | Ovary weight, mg/g |
| Control | 188[a]<br>±8 | 139<br>±25 | 349<br>±178 | 2.4<br>±0.9 | 154<br>±10 | 73<br>±15 | 2538<br>±848 | 34.1<br>±6.1 |
| Control | 202<br>±7 | 169<br>±20 | 2686<br>±297 | 16.0<br>±4.5 | 159<br>±15 | 76<br>±22 | 3609<br>±832 | 51.6<br>±19.5 |
| 0.25 | 183<br>±14 | 125<br>±33 | 439<br>±214 | 3.4<br>±0.8 | 166<br>±16 | 86<br>±22 | 3970<br>±1605 | 46.1<br>±11.4 |
| 0.25 | 196<br>±3 | 157<br>±5 | 1331<br>±219 | 8.5<br>±1.6 | 162<br>±13 | 80<br>±12 | 3132<br>±1458 | 41.5<br>±23.5 |
| 0.54 | 187<br>±11 | 137<br>±27 | 820<br>±640 | 6.2<br>±4.4 | 165<br>±4 | 88<br>±10 | 3920<br>±1700 | 44.4<br>±17.3 |
| 0.54 | 192<br>±6 | 155<br>±18 | 933<br>±263 | 6.2<br>±2.3 | 157<br>±9 | 76<br>±16 | 3515<br>±1604 | 46.3<br>±17.6 |
| 1.22 | 195<br>±5 | 174<br>±9 | 1924<br>±579 | 15.4<br>±7.9 | 156<br>±23 | 78<br>±40 | 4282<br>±4296 | 47.9<br>±23.0 |

Continued ....

TABLE 14. CONDITIONS OF ADULT BLUEGILL AT TERMINATION OF CHRONIC TOXICITY TEST--continued

| Meas. chlordane conc., µg/l | Males |  |  |  | Females |  |  |  |
|---|---|---|---|---|---|---|---|---|
|  | Total length, mm | Wet weight, g | Testes weight, mg | Testes weight, mg/g | Total length, mm | Wet weight, g | Ovary weight, mg | Ovary weight, mg/g |
| 1.22 | 188<br>±7 | 152<br>±24 | 1607<br>±854 | 10.6<br>±5.9 | 165<br>±13 | 86<br>±19 | 4016<br>±2259 | 46.8<br>±25.4 |
| 2.20 | 186<br>±8 | 110<br>±12 | 754<br>±748 | 6.9<br>±7.1 | 176<br>±14 | 93<br>±22 | 3812<br>±2399 | 39.3<br>±21.1 |
| 2.20 | 200<br>±8 | 173<br>±14 | 747<br>±8 | 4.4<br>±0.4 | 155<br>±4 | 67<br>±6 | 1373<br>±487 | 20.7<br>±7.3 |
| 5.17 | 177<br>±9 | 106<br>±24 | 588<br>±446 | 5.0<br>±3.1 | ...[b] | ... | ... | ... |
| 5.17 | ...[b] | ... | ... | ... | ... | ... | ... | ... |

[a] Mean ±1 standard deviation.

[b] All fish had perished.

TABLE 15. SURVIVAL OF $F_1$-GENERATION BLUEGILL IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Initial No. fry | % survival 30 days | % survival 60 days | % survival 90 days |
|---|---|---|---|---|
| Control | 42 | 45.2 | 31.0 | 19.0 |
| Control | 34 | 8.8 | 2.9 | 2.9 |
| Control[a] | 142 | 16.9 | 12.8 | 10.7 |
| 0.25 | 47 | 6.4 | 4.3 | 2.1 |
| 0.25 | 50 | 2.0 | 2.0 | 2.0 |
| 0.25 | 50 | 0 | ... | ... |
| 0.25 | 90 | 3.3 | 3.3 | 3.3 |
| 0.25 | 50 | 4.0 | 4.0 | 4.0 |
| 0.54 | 50 | 0 | ... | ... |
| 0.54[b] | 6 | 33.3 | 33.3 | 33.3 |
| 0.54 | 50 | 12.0 | 12.0 | 12.0 |
| 0.54 | 50 | 0 | ... | ... |
| 0.54 | 44 | 0 | ... | ... |
| 1.22 | 50 | 0 | ... | ... |
| 1.22 | 50 | 4.0 | 4.0 | 4.0 |
| 2.20[c] | 50 | 30.0 | 16.0 | 14.0 |
| 2.20[c] | 50 | 28.0 | 14.0 | 14.0 |
| 2.20[c] | 50 | 2.0 | 0 | ... |
| 2.20[c] | 50 | 10.0 | 2.0 | 2.0 |
| 5.17[c] | 50 | 0 | ... | ... |
| 5.17[c] | 50 | 0 | ... | ... |

[a] Fry transferred from 0.25 µg/l concentration.

[b] Fry from eggs incubated in control.

[c] Fry hatched from control eggs.

## TABLE 16. GROWTH OF $F_1$-GENERATION BLUEGILL DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Growth | | | | | | |
|---|---|---|---|---|---|---|---|
| | 30 days | | 60 days | | 90 days | | |
| | No. fry | Total length, mm | No. fry | Total length, mm | No. fry | Total length, mm | Wet weight, g |
| Control | 23 | 6.4[a]<br>±0.6 | 14 | 9.9<br>±0.9 | 9 | 17.3<br>±3.9 | 107<br>±35 |
| Control | 24 | 11.1<br>±1.8 | 18 | 12.1<br>±1.7 | 15 | 16.9<br>±2.0 | 64<br>±28 |
| 0.25 | 8 | 13.0<br>±0.6 | 8 | 28.0<br>±2.4 | 7 | 36.8<br>±3.3 | 752<br>±206 |
| 0.54 | 8 | 14.0<br>±2.6 | 8 | 24.8<br>±2.4 | 8 | 32.8<br>±4.0 | 481<br>±198 |
| 1.22 | 2 | 11.5 | 2 | 27.5 | 2 | 36.0 | 760 |
| 2.20[b] | 35 | 6.7<br>±0.6 | 16 | 14.9<br>±3.5 | 15 | 18.7<br>±6.5 | 132<br>±88 |

[a] Mean ±1 standard deviation are given.

[b] Fry hatched from control embryos.

As was observed in the chronic tests of the other fish species, measured chlordane levels were 42 - 58% of desired and ranged from 0.32 ± 0.18 to 5.80 ± 2.15 µg/l (Appendix Table 15).

Chronic Effects on Survival, Growth and Reproduction

Growth of $f_o$-generation brook trout throughout the chronic test did not vary significantly between treatments in terms of either total length or wet body weight, although fish exposed to 2.21 and 5.80 µg/l chlordane tended to be smaller at 3 months. At the beginning of the test, the trout averaged 188 mm in total length and 70 g in wet weight. After 6.5 months, they had increased 25% in length (to 248 mm) and more than doubled their body weight (188 g). At termination average lengths and weights were 213 mm and 281 g, respectively (Tables 17 and 18).

Prior to and during spawning, mortality was much higher among fish exposed to 2.21 and 5.80 µg/l than among controls or those exposed to the lower (0.32 - 1.29 µg/l) concentrations (Table 19). For trout exposed to 5.8 µg/l chlordane, mortality was 91.7% after 6.5 months' exposure and complete at the conclusion of spawning. Control trout, which had been transferred to one of the 5.8 µg/l tanks at thinning (the sole fish remaining being transferred to the other tank), also died during spawning. Interestingly, about half of the fish which perished had signs characteristic of bacterial hemorrhagic septicemia (e.g. exophthalmia and lesions). Those dying from what was believed to be chlordane poisoning were emaciated and most exhibited impaired equilibrium for up to several weeks before death. Convulsive or other behavior indicative of poisoning by some insecticides was not observed.

After 8 months' exposure (28 November 1973), trout began spawning. Total embryo production per female ranged from 62 to 400 in the control, 0.32, 0.66, and 1.29 µg/l concentrations, but only from 0 to 47 in the 2.21 and 5.80 µg/l concentrations (Table 20). Trout exposed to the two highest chlordane concentrations also spawned fewer than 100 embryos at any time, whereas spawns greater than 100 embryos each comprised from 9.1 to 26.9% of the total spawns in the lower pesticide concentrations and the controls.

At the time spawnings were checked, i.e. within 24 hr of spawning, the proportions of dead (opaque) embryos were greater at higher pesticide concentrations (Table 20). On the average, only 8.0% of the embryos produced by controls were dead, compared to 14.8, 23.4, and 67.5% in the low, mid-range, and high concentrations, respectively.

Spawns totalling more than 20 embryos were used for determination of viability. Embryo viability was lower at higher technical chlordane concentrations, although embryos produced by one of the control tanks and one of the 0.66 µg/l tanks were nonviable. Average embryo viabilities declined from 65% in the controls to 47% in the 0.32 µg/l concentration to 17% in the 0.66 and 1.29 µg/l concentrations (Table 21). None of the embryos produced in the 2.21 and 5.80 µg/l concentrations were viable. Four lots of 50

TABLE 17. TOTAL LENGTHS OF $F_0$-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Total length, mm | | | |
|---|---|---|---|---|
| | 0 month | 3 months | 6.5 months | 12 months |
| Control | 188[a]<br>±8 | 211<br>±10 | 250<br>±12 | 285<br>±17 |
| Control | 185<br>±11 | 208<br>±14 | 243<br>±16 | 281<br>±22 |
| 0.32 | 187<br>±13 | 208<br>±14 | 249<br>±16 | 278<br>±33 |
| 0.32 | 192<br>±9 | 211<br>±10 | 250<br>±10 | 286<br>±19 |
| 0.66 | 188<br>±15 | 210<br>±15 | 246<br>±21 | 286<br>±31 |
| 0.66 | 187<br>±13 | 209<br>±14 | 243<br>±22 | 286<br>±11 |
| 1.29 | 190<br>±15 | 210<br>±13 | 252<br>±16 | 285<br>±11 |
| 1.29 | 195<br>±11 | 213<br>±12 | 254<br>±15 | 288<br>±14 |
| 2.21 | 187<br>±10 | 195<br>±21 | 257[b]<br>... | ...[b] |
| 2.21 | 186<br>±13 | 203<br>±17 | 247<br>±21 | 279<br>±34 |
| 5.80 | 188<br>±9 | 209<br>±10 | 241[b]<br>... | ...[b] |
| 5.80 | 187<br>±15 | 199<br>±27 | 210[b]<br>... | ...[b] |

[a] Mean ±1 standard deviation.

[b] One or no fish remaining.

## TABLE 18. BODY WEIGHTS OF $F_0$-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Wet body weight, g | | | |
|---|---|---|---|---|
| | 0 month | 3 months | 6.5 months | 12 months |
| Control | 69[a]<br>±12 | 106<br>±18 | 190<br>±33 | 288<br>±65 |
| Control | 68<br>±12 | 103<br>±13 | 177<br>±34 | 268<br>±94 |
| 0.32 | 68<br>±14 | 107<br>±20 | 192<br>±42 | 301<br>±78 |
| 0.32 | 72<br>±12 | 105<br>±16 | 190<br>±22 | 276<br>±70 |
| 0.66 | 72<br>±18 | 107<br>±21 | 186<br>±53 | 297<br>±149 |
| 0.66 | 67<br>±15 | 104<br>±23 | 181<br>±50 | 278<br>±40 |
| 1.29 | 74<br>±15 | 107<br>±15 | 196<br>±31 | 291<br>±38 |
| 1.29 | 77<br>±13 | 110<br>±16 | 198<br>±36 | 286<br>±59 |
| 2.21 | 71<br>±14 | 90<br>±38 | 210[b]<br>... | ...[b] |
| 2.21 | 68<br>±16 | 97<br>±25 | 179<br>±57 | 247<br>±100 |
| 5.80 | 69<br>±10 | 96<br>±17 | 157[b]<br>... | ...[b] |
| 5.80 | 70<br>±17 | 96<br>±31 | 107[b]<br>... | ...[b] |

[a] Mean ±1 standard deviation.

[b] One or no fish remaining.

## TABLE 19. MORTALITY OF $F_0$-GENERATION BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Cumulative mortality[a], % |  | Mortality during spawning[b], 6.5 - 12 mo |  |
|---|---|---|---|---|
|  | 3 mo | 6.5 mo (up to thinning) | Males | Females |
| Control | 0 | 8 | 1/3 | 1/4 |
| Control | 0 | 0 | 0/3 | 0/4 |
| 0.32 | 8 | 17 | 0/3 | 0/4 |
| 0.32 | 0 | 17 | 0/3 | 0/4 |
| 0.66 | 0 | 17 | 0/2 | 0/5 |
| 0.66 | 0 | 8 | 0/3 | 0/4 |
| 1.29 | 8 | 17 | 0/3 | 0/4 |
| 1.29 | 8 | 17 | 0/2 | 0/5 |
| 2.21 | 67 | 92 | 1/3 | 1/2 |
| 2.21 | 0 | 17 | 3/4 | 1/2 |
| 5.80 | 42 | 92 | 3/3[c] | 4/4 |
| 5.80 | 83 | 92 | 0/0 | 2/2 |

[a] Based on 12 fish per chamber.

[b] Numerator and denominator of each expression equivalent to number of fish dying out of total, i.e. 1/3 (male column) indicates one of three males died during spawning.

[c] Represent control fish transferred at thinning. The fish which had survived to thinning in this treatment was transferred to the other replicate.

## TABLE 20. SPAWNING SUCCESS OF BROOK TROUT CHRONICALLY EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | No. females | Embryos/ female | No. embryos/spawn ≥ 1 | >20 | >100 | % embryos initially dead |
|---|---|---|---|---|---|---|
| Control | 4 | 290 | 22 | 11 | 4 | 7.3 |
| Control | 4 | 90 | 9 | 3 | 1 | 10.1 |
| 0.32 | 4 | 400 | 29 | 14 | 4 | 13.9 |
| 0.32 | 4 | 62 | 15 | 4 | 0 | 21.1 |
| 0.66 | 5 | 215 | 16 | 12 | 5 | 25.3 |
| 0.66 | 4 | 153 | 10 | 6 | 2 | 5.1 |
| 1.29 | 4 | 259 | 14 | 5 | 4 | 6.6 |
| 1.29 | 5 | 126 | 29 | 8 | 1 | 50.9 |
| 2.21 | 2 | 47 | 10 | 1 | 0 | 40.9 |
| 2.21 | 2 | 30 | 5 | 1 | 0 | 13.3 |
| 5.80 | 4 | 32 | 16 | 1 | 0 | 67.5 |
| 5.80 | 2 | 0 | ... | ... | ... | ... |

TABLE 21. VIABILITY AND HATCH OF EMBRYOS AND CONDITIONS OF $F_1$-GENERATION BROOK TROUT ALEVINS

| Meas. conc., chlordane, µg/l | Viability | | | Hatching success | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | No. embryos incubated | % viable embryos | | No. embryos incubated | % alevins | | | % hatch | |
| | | tank | conc. | | normal | abnormal | dead | tank | conc. |
| Control | 970 | 82.0 | 65.4[a] | 450 | 97.3 | 2.4 | 0.3 | 91.8 | 91.8 |
| Control | 247 | 0 | | ... | ... | ... | ... | ... | |
| 0.32 | 1,256 | 42.7 | 46.7 | 200 | 92.9 | 4.7 | 2.4 | 42.5 | 59.7 |
| 0.32 | 154 | 79.8 | | 100 | 100.0 | 0 | 0 | 94.0 | |
| 0.66 | 734 | 0 | 17.1 | 0 | ... | ... | ... | ... | 90.0 |
| 0.66 | 567 | 39.3 | | 50 | 93.4 | 4.4 | 2.2 | 90.0 | |
| 1.29 | 941 | 23.1 | 17.4 | 50 | 97.3 | 2.7 | 0 | 74.0 | 74.0 |
| 1.29 | 309 | 0.3 | | 0 | ... | ... | ... | ... | |
| 2.21 | 0 | ... | ... | ... | ... | ... | ... | ... | ... |
| 2.21 | 0 | ... | ... | ... | ... | ... | ... | ... | ... |
| 5.80[b] | 34[c] | 0 | 0 | 0 | ... | ... | ... | ... | ... |
| 5.80 | ... | ... | | ... | ... | ... | ... | ... | |

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[a] Weighted mean.

[b] Control fish transferred at "thinning".

[c] Both fish were females.

embryos were transferred from control chambers to the 2.21 and 5.80 μg/l concentrations, and viabilities for both treatments were as high (80 to 96%) as controls, suggesting that the concentrations employed were not deleterious within the 12-day period following spawning.

Hatching success was largely unaffected by technical chlordane up to concentrations of at least 1.29 μg/l (Table 21). Although too few eggs were spawned by fish reared in the 2.21 and 5.80 μconcentrations to evaluate hatching success, control eggs, incubated in these treatments for the 50 to 55 days (range of median hatch dates for all treatments) required for hatching, survived as well (hatching success of 74 to 98%) as controls. Furthermore, technical chlordane had no effect on the proportions of abnormally developed or dead alevins at hatching (less than 3% in all cases).

Growth of the $f_1$-generation generation progeny was followed over a 90-day period (Table 22). Upon hatching total lengths of subsamples of alevins from each of the treatments were similar. After 30 days' growth, total lengths of fry reared in 0.66, 1.29, 2.21 and 5.80 μg/l tended to be less than controls, but after 60 and 90 days, all chlordane-exposed fry were larger than controls. Although analysis of variance indicated significant differences after all three growth periods ($p < 0.05$), Dunnett's test indicated that the significant differences were between the treatments and not between the treatments and the control. Data on wet body weights suggested similar relationships to those discussed for the total length data (Table 22).

Survival data for fry were incomplete, owing to high mortality in approximately 20- to 40-day-old alevins which occurred on two successive weekends. The cause was traced to chlorination of the water supply on Fridays for removal of algae in storage reservoirs. The water supply to the brook trout chronic test was not being passed through an activated charcoal filter owing to the high volume flow required for this test. Lack of this protection was responsible for the unanticipated mortalities. After the cause of the mortality was identified, a solution of sodium thiosulfate was pumped into the water supply line at a concentration of 100 μg/l to convert chlorine to chloride ion. This eliminated further mortality.

In summary, chronic exposure of brook trout to technical chlordane appeared to cause detrimental effects on survival, embryo production, spawn size, and the viability and hatch of $f_1$-generation progeny. However, the importance of these effects can only be speculated since none were statistically significant ($p > 0.05$). Survival, embryo production, and spawn size were substantially lower than controls in insecticide concentrations greater than 1.29 to 2.21 μg/l. Greater proportions of the embryos spawned were found to be dead down to the lowest concentration tested (0.32 μg/l), while 12-day survival of the embryos was 29% lower in this concentration than in the controls. On the other hand, survival to hatching was reduced only above 1.29 μg/l. From the above data it appears that the lowest concentration employed, 0.32 μg/l, would be deleterious to populations of brook trout.

## TABLE 22. GROWTH OF F$_1$-GENERATION BROOK TROUT DURING CHRONIC EXPOSURE TO TECHNICAL CHLORDANE

| Meas. conc. chlordane, µg/l | Total length, mm | | | | Wet body weight, g | |
|---|---|---|---|---|---|---|
| | At hatch | 30 days | 60 days | 90 days | At hatch | 90 days |
| Control | 14.4[a]<br>±0.6<br>(125) | 21.4<br>±0.8<br>(89) | 28.4<br>±3.5<br>(39) | 39.8<br>±2.9<br>(20) | 0.050<br>±0.005<br>(110) | 0.610<br>±0.144<br>(20) |
| 0.32 | 14.5<br>±0.6<br>(114) | 21.8<br>±0.8<br>(58) | 26.5<br>±1.8<br>(36) | 44.7<br>±3.5<br>(24) | 0.044<br>±0.004<br>(60) | 0.910<br>±0.201<br>(24) |
| 0.66 | 13.8<br>±0.4<br>(25) | 17.2<br>±0.7<br>(24) | 29.6[b]<br>±2.1<br>(25) | 43.4[b]<br>±2.8<br>(14) | 0.041<br>±0.003<br>(19) | 0.803[b]<br>±0.155<br>(14) |
| 1.29 | 14.8<br>±0.4<br>(25) | 19.4<br>±0.6<br>(25) | 29.4[b]<br>±2.0<br>(25) | 43.8[b]<br>±3.1<br>(16) | 0.050<br>±0.002<br>(12) | 0.847[b]<br>±0.169<br>(16) |
| 2.21 | 14.5[b]<br>±0<br>(25) | 18.6[b]<br>±0.9<br>(21) | ... | ... | 0.047[b]<br>±0.004<br>(21) | ... |
| 5.80 | 14.6[b]<br>±0.6<br>(75) | 17.4[b]<br>±0.7<br>(71) | ... | ... | 0.050[b]<br>±0.006<br>(57) | ... |

[a] Means ± 1 standard deviation and sample size are given.

[b] Control eggs that had been transferred to these concentrations.

# CHRONIC TOXICITY TO *HYALLELA AZTECA*

## Water Quality and Chlordane Concentrations

Water quality was measured eight times during the 9-week chronic test utilizing *H. azteca* (Appendix Table 16). Water temperatures averaged 16.7 ± 1.0°C and dissolved oxygen, 7.5 ± 0.4 mg/l (76% of air saturation). As in all toxicity tests, the water was alkaline (pH of 7.86) and of intermediate hardness (148 mg/l). Fluctuations from one week to the next were small.

Measured chlordane concentrations were approximately 50% of desired, averaging 1.41 ± 0.77, 2.64 ± 1.32, 5.32 ± 3.24, 11.53 ± 6.14, and 20.52 ± 9.85 µg/l (Appendix Table 17).

## Toxicity

Two of the main indices of chlordane's chronic effects on *H. azteca*, namely growth and survival, were determined at the end of the experiment. Use of only one point of measurement rather than several was selected because the responses of the animals to handling were unknown. Adult *H. azteca* are about one-quarter to one-third the size of *Gammarus pseudolimnaeus*, the species for which this test was patterned; accordingly, it was presumed that much greater care would be required during handling.

Survival of *H. azteca* was unaffected at technical chlordane concentrations less than 11.5 µg/l, where 92% or more of the specimens survived 9 weeks relative to 88 - 108% of controls (Table 23). However, survival was significantly reduced to 12 - 36% in the 11.5 µg/l concentration and to zero in the 20.5 µg/l level.

Growth of the amphipods was also affected by the presence of chlordane. In both replicates, analysis of variance and Dunnett's test indicated that amphipods exposed to 11.5 µg/l chlordane were significantly smaller ($p < 0.05$) than controls in terms of wet and dry weights (Table 23 and Appendix Table 18).

The chronic toxicity test indicated that growth and survival of *H. azteca* were significantly reduced between concentrations of 5.3 and 11.5 µg/l. The maximum acceptable toxicant concentration for technical chlordane may exist within this range, but effects of this insecticide on reproduction and on growth and survival of progeny should be examined before arriving at this conclusion.

## Accumulation of Chlordane

Contents of heptachlor, β-, γ- and "a"-chlordane, *cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor were determined on a dry weight basis in *H. azteca* at the conclusion of the chronic test at 65 days. Contents of each constituent increased with aqueous concentration. Concentration factors tended to remain unaffected by the level of treatment for

TABLE 23. RELATIVE SURVIVAL AND GROWTH OF HYALLELA AZTECA EXPOSED TO TECHNICAL CHLORDANE

| Parameter | Measured concentration of technical chlordane, µg/l | | | | | |
|---|---|---|---|---|---|---|
| | Control | 1.4 | 2.6 | 5.3 | 11.5 | 20.5 |
| Replicate 1 | | | | | | |
| No. survivors[a] | 27 | 23 | 23 | 24 | 3 | 0 |
| % survivors | 108 | 92 | 92 | 96 | 12 | 0 |
| Wet body weight, mg[b] | 6.3<br>±1.3 | 6.2<br>±1.5 | 6.4<br>±1.2 | 5.1<br>±0.9 | 3.8<br>±0.7 | ...<br>... |
| Dry weight, mg[b] | 1.58 | 1.49 | 1.57 | 1.37 | 0.87 | ... |
| Replicate II | | | | | | |
| No. survivors | 22 | 25 | 25 | 24 | 9 | 0 |
| % survivors | 88 | 100 | 100 | 96 | 36 | 0 |
| Wet body weight, mg[b] | 7.5<br>±1.3 | 5.8<br>±1.3 | 5.8<br>±1.6 | 5.5<br>±1.6 | 5.3<br>±1.0 | ...<br>... |
| Dry weight, mg[b] | 1.92 | 1.55 | 1.53 | 1.35 | 1.33 | ... |

[a] 25 individuals introduced initially per chamber.

[b] Average calculated weight per individual.

heptachlor, the chlordenes, and *cis*-nonachlor, and increased only slightly for *cis*-chlordane, *trans*-chlordane and *trans*-nonachlor. The highest contents of each constituent were 357 μg/g for heptachlor, 92.3 μg/g for the chlordenes, 260 μg/g for *trans*-chlordane, 220 μg/g for *cis*-chlordane, 71.8 μg/g for *trans*-nonachlor, and 46.4 μg/g for *cis*-nonachlor (Table 24).

Net concentration of all constituents was very extensive. Even though heptachlor was concentrated to a lesser extent than the other compounds, its concentration factors were still quite high (16,700 to 31,040). Although the *cis*- and *trans*-nonachlors comprised only 2.8 and 5.1% of the technical chlordane, their storage was proportionally greater than all other major constituents including the *cis*- and *trans*-chlordanes, which comprised 43% of the insecticide. The compound accumulated to the greatest extent was *cis*-nonachlor, for which concentration factors ranging from 95,030 to 144,100 were calculated. The propensity of the components to be concentrated increased in the following order: heptachlor, the chlordenes, *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor, and *cis*-nonachlor (Table 24).

The proportions of the chlordane constituents present in the amphipods were different from those characterizing the neat insecticide, suggesting differences in water solubility, uptake, or metabolism. For example, the ratio of *cis*- *trans*-nonachlor in the neat technical insecticide was 0.55:1, but the average ratio in the organisms was 0.71:1, indicating an enhanced accumulation of *cis*-nonachlor relative to the *trans* isomer. A preferential storage of *cis*-chlordane relative to that of the *trans* isomer was also apparent, with the mean ratio of *cis*- *trans*-chlordane of 0.85:1 in the tissues being somewhat greater than that (0.79:1) characterizing the neat insecticide. There was also a diminution in heptachlor storage relative to the two chlordanes, for the ratio in the tissues (0.07:1) was only 32% of that in the stock formulation (0.23:1). Considerably more *cis*- and *trans*-nonachlor were stored relative to *cis*- and *trans*-chlordane. The ratio of chlordanes to nonachlors was 4.1:1 in amphipod tissues and 5.4:1 in the neat insecticide. Although these studies were not intended to elucidate the *in vivo* fate of the various chlordane components, it appears that the nonachlors may have been particularly susceptible to uptake and deposition or were generated in part through metabolism of such constituents as *cis*- and *trans*-chlordane or heptachlor, which were present in diminished proportions in amphipod tissues.

The relative proportions of some of the constituents also appeared to change as a function of aqueous technical chlordane concentration. For example, the heptachlor/*cis*- and *trans*-chlordane ratio declined from 0.085:1 in specimens exposed to 1.4 μg/l technical chlordane to 0.063:1 in those exposed to 11.5 μg/l, while the *cis*-/*trans*-nonachlor ratio declined from 0.83:1 in amphipods exposed to 1.4 μg/l to 0.59:1 in those exposed to 11.5 μg/l. In contrast, the contents of the chlordanes relative to the nonachlors may have increased since the ratio between them was slightly greater (4.2:1) in the two highest concentrations than in the two lowest (3.76:1 and 3.79:1). The *cis*-/*trans*-chlordane ratio was essentially constant between treatments (0.83:1 to 0.87:1). These differences suggest that amphipods which were exposed to the higher technical chlordane concentrations

TABLE 24. CONTENTS AND CONCENTRATION FACTORS (C.F.) OF CHLORDANE CONSTITUENTS IN DRIED *HYALLELA AZTECA* THAT HAD BEEN EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane | Heptachlor | | Chlordenes[a] | | *Cis*-chlordane | | *Trans*-chlordane | | *Cis*-nonachlor | | *Trans*-nonachlor | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| conc., µg/l | Content, µg/g[b] | C.F. | Content, µg/g | C.F. | Content, µg/g | C.F. | Content, µg/g | C.F. | Content, µg/g | C.F. | Content, µg/g | |
| Control | 0.3 | ...[c] | 1.4 | ... | 1.9 | ... | 2.2 | ... | 0.2 | ... | 0.5 | ... |
| Control | 0.5 | ... | 1.4 | ... | 2.7 | ... | 2.9 | ... | 0.6 | ... | 0.5 | ... |
| 1.4 | 3.4 | 24,290 | 10.1 | 55,500 | 19.2 | 72,180 | 22.1 | 65,770 | 5.3 | 135,200 | 6.1 | 85,430 |
| 1.4 | 3.1 | 22,140 | 8.2 | 45,060 | 16.2 | 60,900 | 18.7 | 55,660 | 3.8 | 96,940 | 4.9 | 68,630 |
| 2.6 | 5.3 | 20,390 | 17.8 | 52,660 | 36.9 | 74,700 | 42.5 | 68,110 | 9.2 | 126,370 | 11.8 | 88,990 |
| 2.6 | 5.8 | 22,310 | 17.7 | 52,370 | 35.5 | 71,860 | 41.3 | 66,190 | 9.1 | 125,000 | 11.4 | 85,970 |
| 5.3 | 10.9 | 20,570 | 29.6 | 42,960 | 73.5 | 72,990 | 88.6 | 69,650 | 14.9 | 100,400 | 23.2 | 85,830 |
| 5.3 | 11.7 | 22,080 | 32.6 | 47,320 | 81.7 | 81,130 | 97.4 | 76,572 | 16.8 | 113,210 | 26.1 | 96,560 |
| 11.5 | 19.2 | 16,700 | 60.7 | 40,600 | 176.9 | 80,960 | 216.0 | 78,261 | 30.6 | 95,030 | 58.9 | 100,430 |
| 11.5 | 35.7 | 31,040 | 92.3 | 61,740 | 220.0 | 100,690 | 259.9 | 94,170 | 46.4 | 144,100 | 71.8 | 122,420 |

[a] Consisting of $\gamma$ - $\beta$ peaks (13) and "a" peak (12).

[b] µg/g residue per dry body weight.

[c] Concentration factors (C.F.) not calculated for controls since on only one occasion was technical chlordane detected.

may have dealt with the various constituents differently than those exposed to lesser concentrations.

## CHRONIC TOXICITY TO DAPHNIA MAGNA

### Water Quality and Measured Chlordane Concentrations

The standard battery of water quality parameters was measured four times, just prior to and during the course of the chronic toxicity test using *D. magna*. The water temperature averaged 20.9 ± 0.5°C and the water quality was very similar to that described earlier for the other chronic toxicity tests (Appendix Table 19).

Desired concentrations of technical chlordane ranged from 6.1 to 96.9 µg/l, excluding the control, and were set high because of excessive loss of the insecticide under the conditions of limited toxicant renewal. The concentrations existing during the test ranged from 1.7 ± 0.1 to 21.6 ± 9.6 µg/l for the five treatments (Appendix Table 20).

### Effects on Survival, Growth and Reproduction

Survival of the cladocerans from first instars to adults was poor regardless of treatment. After 1 week, control survival averaged 80%, but declined to 30% in the fourth week (Table 25). Survival of daphnids in chlordane was essentially the same as that of the controls, except for those exposed to 21.6 µg/l chlordane, where only one of the initial 20 specimens survived to the middle of the fourth week.

Growth of the cladocerans was determined only for instars produced in the fourth week. Since dry weights of instars produced in the various chlordane solutions were commensurate with those for controls, there did not appear to be any adverse effects upon growth (Table 26).

Reproduction of *Daphnia* was highly variable and was apparently unaffected by any of the concentrations of technical chlordane used (Table 25).

On the basis of the above data, which should be considered preliminary pending completion of experiments having good control survival and reproduction, it appears that the only toxicologically effective concentration was 21.6 µg/l, which had killed all first generation daphnids by the end of the fourth week.

### Accumulation of Chlordane

Accumulation of the major components of chlordane in *D. magna* was similar in magnitude to that of *H. azteca*, even though the cladocerans were exposed for a maximum of 1 week[2] and the amphipods for 2 months. Uptake and storage are evidently very rapid in *D. magna*.

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[2] First instars produced in the last 7 days of the chronic test were used for for residue analysis.

## TABLE 25. SURVIVAL AND REPRODUCTION OF *DAPHNIA MAGNA* IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

| Week | Meas. chlordane conc., µg/l | Adult survival,[a] % | Production | |
|---|---|---|---|---|
| | | | Total instars | Instars/avg. No. adults |
| 5/8/74 | Control | 80 | 0 | 0 |
| | Control | 80 | 0 | 0 |
| | 1.7 | 90 | 0 | 0 |
| | 1.7 | 80 | 0 | 0 |
| | 2.5 | 70 | 0 | 0 |
| | 2.5 | 80 | 0 | 0 |
| | 6.2 | 60 | 0 | 0 |
| | 6.2 | 70 | 0 | 0 |
| | 12.1 | 90 | 0 | 0 |
| | 12.1 | 90 | 0 | 0 |
| | 21.6 | 50 | 0 | 0 |
| | 21.6 | 100 | 0 | 0 |
| 5/15/74 | Control | 50 | 25 | 5 |
| | Control | 40 | 16 | 4 |
| | 1.7 | 80 | 13 | 2 |
| | 1.7 | 70 | 7 | 1 |
| | 2.5 | 40 | 13 | 3 |
| | 2.5 | 70 | 34 | 5 |
| | 6.2 | 20 | 7 | 4 |
| | 6.2 | 50 | 13 | 3 |
| | 12.1 | 80 | 39 | 5 |
| | 12.1 | 80 | 27 | 3 |
| | 21.6 | 0 | 0 | 0 |
| | 21.6 | 90 | 36 | 4 |
| 5/23/74 | Control | 40 | 12 | 3 |
| | Control | 30 | 22 | 7 |
| | 1.7 | 70 | 176 | 25 |
| | 1.7[b] | 40 | 3 | 1 |
| | 2.5 | 40 | 28 | 7 |
| | 2.5 | 70 | 334 | 48 |
| | 6.2 | 20 | 28 | 14 |
| | 6.2 | 50 | 91 | 18 |
| | 12.1 | 70 | 74 | 11 |
| | 12.1 | 80 | 220 | 28 |
| | 21.6 | 0 | 0 | 0 |
| | 21.6 | 40 | 15 | 3 |

Continued.....

TABLE 25. SURVIVAL AND REPRODUCTION OF *DAPHNIA MAGNA* IN CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE--continued

| Week | Meas. chlordane conc., µg/l | Adult survival,[a] % | Production | |
|---|---|---|---|---|
| | | | Total instars | Instars/avg. No. adults |
| 5/30/74 | Control | 40 | 115 | 29 |
| | Control | 20 | 57 | 29 |
| | 1.7 | 60 | 804 | 134 |
| | 1.7 | 0 | ... | ... |
| | 2.5 | 40 | 215 | 54 |
| | 2.5 | 70 | 619 | 88 |
| | 6.2 | 20 | 162 | 81 |
| | 6.2 | 50 | 166 | 33 |
| | 12.1 | 60 | 143 | 24 |
| | 12.1 | 70 | 791 | 105 |
| | 21.6 | 0 | 0 | 0 |
| | 21.6 | 0 | 85 | 170 |

[a] Ten *Daphnia* initially introduced into each test container. Values represent percentage of specimens remaining at the end of a given week.

[b] Accidental discard of remaining adults in this concentration.

### TABLE 26. AVERAGE DRY BODY WEIGHTS OF FIRST INSTAR *DAPHNIA MAGNA* PRODUCED DURING FOURTH WEEK OF CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | No. specimens | Average dry weight/individual, µg |
|---|---|---|
| Control | 115 | 49.1 |
| Control | 57 | 27.8 |
| 1.7 | 804 | 31.2 |
| 1.7 | 0 | ... |
| 2.5 | 215 | 36.7 |
| 2.5 | 619 | 29.5 |
| 6.2 | 162 | 28.3 |
| 6.2 | 166 | 23.4 |
| 12.1 | 143 | 24.4 |
| 12.1 | 791 | 27.5 |
| 21.6 | 0 | ... |
| 21.6 | 85 | 43.5 |

As shown in Table 27, which gives the actual tissue levels of the various components as well as their individual concentration factors, heptachlor residues were lowest (range of 0.9 - 27.9 μg/g) and *trans*-chlordane residues highest (9.6 - 370 μg/g) of the six components selected for analysis. Daphnids tended to preferentially concentrate more *cis*-and *trans*-nonachlor and less heptachlor and chlordenes than *cis*- or *trans*-chlordane. Tissue residues of each compound appeared to be directly proportional to the aqueous concentration of technical chlordane to which the animals were exposed. The linearity of uptake and storage was corroborated by the uniform factors for each component, which varied little as a function of treatment, except for the low aqueous concentration, where concentration factors were considerably lower. Concentration factors varied from 5,290 to 12,900 for heptachlor to 32,000-144,850 for *trans*-nonachlor. The order of increasing bioconcentration was: heptachlor, the chlordenes, *trans*-chlordane, *cis*-chlordane, *cis*-nonachlor, and *trans*-nonachlor.

As was observed for amphipods, the relative proportions of the chlordane constituents differed from those characterizing the neat insecticide. There was preferential deposition of the *cis*- and *trans*-nonachlors and a relative diminution of heptachlor and the *cis*- and *trans*-chlordanes.

## CHRONIC TOXICITY TO *CHIRONOMUS* NO. 51

Two partial chronic toxicity tests were conducted with *Chironomus* No. 51 to delimit "safe" and "unsafe" concentrations. The first test was preliminary, limited to introduction of 25 newly-hatched larvae into each of five insecticide concentrations and a control. The second test utilized 50 newly-hatched larvae and 12 test chambers, comprising five concentrations and a control in duplicate.

### Water Quality and Chlordane Concentrations

Levels of each of the water quality parameters comprising the standard battery were essentially the same in both experiments. Concentrations of dissolved oxygen were 80% of air saturation, pH levels averaged 7.94 and 7.90, and total hardness averaged 154 and 150 mg/l $CaCO_3$, respectively (Appendix Table 21).

Measured concentrations of technical chlordane ranged from 1.0 ± 0.1 to 24.9 ± 16.8 μg/l in the first test and from 0.7 ± 0.1 to 15.5 ± 3.7 μg/l in the second (Appendix Table 22).

### Toxicity

Adult *Chironomus* No. 51 emerged in the first test 11 to 15 days after their introduction as newly-hatched larvae. All control larvae and those held in 1.0 μg/l emerged, but none of those reared in 2.4 to 24.9 μg/l did. The time to 50% adult emergence was 12.5 to 13 days for both treatments (Table 28). Males, which constituted only 28-32% of all adults, tended to emerge 1 day earlier (50% emergence in 11 days) than females.

TABLE 27. CONTENTS AND CONCENTRATION FACTORS (C.F.) OF CHLORDANE CONSTITUENTS IN DRIED *DAPHNIA MAGNA* THAT HAD BEEN EXPOSED TO TECHNICAL CHLORDANE

| Meas. chlordane conc., µg/l | Heptachlor Content, µg/g[b] | Heptachlor C.F. | Chlordenes[a] Content, µg/g | Chlordenes[a] C.F. | *Cis*-chlordane Content, µg/g | *Cis*-chlordane C.F. | *Trans*-chlordane Content, µg/g | *Trans*-chlordane C.F. | *Cis*-nonachlor Content, µg/g | *Cis*-nonachlor C.F. | *Trans*-nonachlor Content, µg/g | *Trans*-nonachlor C.F. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 0.5 | ...[c] | 5.7 | ... | 6.8 | ... | 6.7 | ... | 1.5 | ... | 1.6 | ... |
| Control | 0.5 | ... | 4.3 | ... | 6.1 | ... | 6.0 | ... | 1.4 | ... | 1.5 | ... |
| 1.7 | 0.9 | 5,290 | 4.8 | 21,720 | 8.9 | 27,550 | 9.6 | 23,530 | 2.2 | 46,220 | 2.8 | 32,300 |
| 1.7[d] | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |
| 2.5 | 2.3 | 9,200 | 8.5 | 26,150 | 27.7 | 58,320 | 29.5 | 49,170 | 5.4 | 77,140 | 9.7 | 76,080 |
| 2.5 | 1.9 | 7,600 | 9.2 | 28,310 | 26.6 | 56,000 | 29.8 | 49,670 | 5.9 | 84,290 | 7.7 | 60,390 |
| 6.2 | 3.0 | 4,840 | 11.8 | 14,640 | 71.8 | 60,950 | 77.0 | 51,750 | 16.7 | 96,200 | 27.8 | 87,920 |
| 6.2 | 9.8 | 15,810 | 36.9 | 45,780 | 126.0 | 106,960 | 135.6 | 91,129 | 30.4 | 175,120 | 45.8 | 144,850 |
| 12.1 | 7.4 | 6,120 | 26.1 | 16,590 | 124.3 | 54,070 | 137.5 | 47,350 | 28.4 | 83,830 | 44.2 | 71,630 |
| 12.1 | 9.8 | 8,100 | 31.6 | 20,089 | 152.0 | 66,120 | 168.0 | 57,850 | 29.6 | 87,370 | 62.7 | 101,600 |
| 21.6 | 27.9 | 12,920 | 72.5 | 25,820 | 333.8 | 81,340 | 369.8 | 71,340 | 52.2 | 86,310 | 125.5 | 113,930 |
| 21.6[d] | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... | ... |

[a] Consisting of $\gamma - \beta$ peaks (13) and "a" peak (12).

[b] µg/g dry body weight.

[c] Concentration factors not calculated for controls since technical chlordane was undetected.

[d] No instars produced in fourth week.

TABLE 28. CHRONIC EFFECTS OF TECHNICAL CHLORDANE ON *CHIRONOMUS* NO. 51

| | Test 1 | | | Test 2 | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameter | Control | 1.0 µg/l | 2.4 µg/l | Control | Control | 0.7 µg/l | 0.7 µg/l | 1.7 µg/l | 1.7 µg/l |
| Total adult emergence | 25 | 25 | 0 | 11 | 24 | 10 | 11 | 0 | 0 |
| % emergence | 100 | 100 | 0 | 22 | 48 | 20 | 22 | 0 | 0 |
| % males | 33 | 28 | ... | 63 | 63 | 44 | 36 | ... | ... |
| % females | 67 | 72 | ... | 37 | 37 | 56 | 64 | ... | ... |
| Median emergence time, days | 13 | 12.5 | ... | 13 | 16 | 17 | 15 | ... | ... |

In the second experiment, adult midges emerged only from the control and low concentration (0.7 μg/l) and were not observed in the 1.7 to 15.5 μg/l treatments (Table 28). Although adults were observed on the same day (day 11) as in the first test, emergence was inexplicably spread over a longer period (11 to 25 days) and median times to emergence were longer. In the two control chambers, 50% of the adults had emerged 12 and 16 days, respectively after their introduction as larvae. Fifty percent of the midges exposed to 0.7 μg/l chlordane emerged after 17 and 15 days.

Survival to emergence was much poorer in the second experiment than in the first. Of the 50 larvae originally introduced into each chamber, only 22-48% of the controls and 22-24% of those exposed to 0.7 μg/l chlordane survived. Larvae were observed 4 days after introduction in the 1.7 μg/l concentration, but not thereafter. There was no evidence that chironomids survived for even a short time in the 3.3 to 15.5 μg/l concentrations.

In contrast to the first experiment where females predominated, more males (60 to 63%) emerged in the controls than in the 0.7 μg/l concentration (36 to 44%) in the second test. Males tended to emerge earlier in the control chambers than females, but in the 0.7 μg/l concentration, they emerged at the same rate. Of the first 50% of the control midges to emerge, 80 to 92% were males.

On the basis of the results of the two experiments, technical chlordane concentrations above 0.7 to 1.0 μg/l, i.e. 1.7 μg/l, were clearly unsafe because they were lethal to the developing larvae. Complete life cycle tests encompassing reproductive and hatching success, etc, would be needed to ascertain whether lower levels are deleterious.

## Accumulation of Chlordane

No residues of technical chlordane were detected in extracts of dried adult *Chironomus* No. 51 and there was no evidence of the presence of oxychlordane.

# SECTION VII

# DISCUSSION

## ACUTE TOXICITY TESTS

Chlordane appears to be generally less toxic in the short-term to freshwater fish and invertebrates than endrin, dieldrin, aldrin, and DDT, but more toxic than methoxychlor, lindane, benzene hexachloride (BHC) and Guthion$^R$. In the present study continuously-renewed solutions of technical chlordane were lethal in 96 hr to three fish species between 37 and 59 µg/l. These values agree well with the data of Katz (23), Henderson et al. (22), and of Macek et al. (25), but are consistently higher than values reported by Konar (26) and lower than those given by Lüdemann and Neumann (30). For example, Katz (23) reported 96-hr LC50 estimates of 44 to 57 µg/l for three species of salmonids. Our estimate of 47 µg/l for brook trout was within this range. The 96-hr LC50 of 77 µg/l at 23.8°C for bluegill reported by Macek et al (25) was somewhat higher than we found (59 µg/l) for the same species, but both of these estimates were above the 16.5 µg/l LC50 estimate given by Henderson et al (22). In general brief exposure to chlordane would appear to be lethal to many fish species within the concentration range of 1 to 100 µg/l. More than half of the toxic responses of fish to chlordane reported in the literature (Table 1) and determined in this project were within this range.

Acute toxicity tests conducted with continuous toxicant renewal would in many cases be expected to result in lower lethal limits than tests conducted without renewal (e.g. static conditions) because toxicant concentrations would not decline due to assimilation by the test organisms or by sorption to debris or to the walls of the test vessel. Use of measured rather than expected insecticide concentrations in estimating median response limits should also improve their validity. While these arguments are valid, the LC50 values obtained by us were not demonstrably lower than those reported by others, as indicated above. Differences between static and flow-through test results for chlordane may come to light when exposures are extended beyond approximately 96 hr. For example, the data of Henderson et al. (22) and of Katz (23) indicate that median lethal thresholds, the concentration at which lethality to 50% of the specimens ceases, were approached or reached within 96 hr for fathead minnow, goldfish (*Carassius auratus*), rainbow trout, and chinook salmon (*Oncorhynchus tshawytscha*). In our studies a median lethal threshold was attained only with fathead minnows, but only after 168 hr; toxicity curves for brook trout and bluegill were linear. The absence of median lethal thresholds for exposures less than 96 hr would be expected if the poison had a cumulative action--which chlordane apparently does--and if toxicant concentrations remained relatively constant for the duration of the test.

Comparison of the toxicity test results for D. magna and H. azteca with those reported in the literature for similar species indicates relatively good agreement. The two 96-hr LC50 values for D. magna of 28 and 35 µg/l were only slightly higher than the 48-hr LC50 of 20 µg/l for the cladoceran Simocephalus serrulatus (6). Hyallela azteca are decidedly less sensitive to chlordane than Gammarus lacustris. The 96-hr LC50 of 26 µg/l for the latter species (35) was only 25% that for H. azteca, which were exposed for a longer (168 hr) period. Although there is a great range in the levels of chlordane reported to be toxic to aquatic invertebrates, their sensitivity to this insecticide appears to be of the same order as that for fish (i.e. acute lethal range of 1 to 100 µg/l).

## CHRONIC TOXICITY TESTS

The lowest aqueous concentration of technical chlordane which we found to have marked deleterious chronic effects was 0.32 µg/l, which lowered brook trout embryo viability. This apparent "unsafe" level for chronic exposure was less than 1% of the 96-hr LC50 for this species. Prominent chronic effects were observed for bluegill and the chironomid at concentrations around 2 µg/l. High mortality prior to and during the spawning period and failure to spawn were the salient responses of bluegill that had been exposed to 2.2 and 5.2 µg/l chlordane. Larval mortality, which accounted for the failure of adult emergence, was the main effect of 1.7 µg/l chlordane on Chironomus No. 51. Of lesser apparent sensitivity were fathead minnows, daphnids, and the amphipod. These species were unaffected by chlordane concentrations lower than about 5 to 10 µg/l.

Although the chronic toxicity tests we conducted produced much needed information on the effect of this insecticide on growth, survival, and reproduction of several fish and invertebrate species, they failed in every case to produce hard, unequivocal data on what concentrations were detrimental and which were not for all major life stages of each species. Consequently, technical chlordane may ultimately be found to be more toxic to these species than reported here. For example, the brook trout and bluegill experiments were partial rather than full life cycle tests because they were begun with yearlings instead of fry or embryos. It is quite conceivable, accepting that chlordane's toxicity is cumulative, that greater effects on the $f_0$-generation would have been observed had younger specimens been used. Further, neither the trout nor bluegill tests fully evaluated effects on $f_1$-generation progeny. In both experiments, poor survival compromised interpretation of results. The most notable liability of the fathead minnow chronic was the poor early survival of the fry. As stated earlier, the question of whether the surviving fish were in a weakened condition will remain, even though there is the possibility that the survivors represented the most fit individuals because the weaker fish were selected out. Similarly, poor survival--and hence reproduction--of $f_0$-generation daphnids made conclusions tenuous on the extent of effects. Finally, while the chironomid and amphipod tests went well, they did not evaluate toxicant effects on the $f_1$-generation.

Chronic toxicity, life cycle tests represent an important advance in the sophistication and sensitivity of aquatic toxicity testing. Though costly and time-consuming, they are probably the best means for directly estimating cumulative, long-term effects on most developmental stages of an organism. Because emphasis is placed, when possible, on the results of such tests in setting water quality standards, it is important that the tests be standardized to some extent to insure maximum utility and validity of results. This standardization could include improvements in test conditions to achieve better and more uniform control of specimen quality, identification of key response parameters, and recommendations as to acceptable statistical analyses. For the full potential of the statistics to be realized, several of the chronic tests require better design. Most notable is the need for additional replicates for assessing the various responses of the $f_0$-generation. Increasing the replicates from two to perhaps four, for example, would increase the within-treatment degrees of freedom from 1 to 3 (for a simple one-way analysis of variance). With the present design, differences often have to be rather astounding to be significant because there is considerable within-treatment variation. Also, it would be desirable to stipulate minimum replication for the viability-hatching success determinations since there is the possibility that more data will be accumulated than necessary. Since few laboratories have resident biometricians, information could be wasted if the experimental design were left solely to the discretion of the investigator. This would be particularly true for the first few tests conducted.

In addition to the fundamental changes suggested above, there are specific changes in methodology which should be considered for each of the organisms we tested. The apparent lack of fertilization of spawned eggs in the trout tests was anomalous and should be studied further. It was also encountered in three other brook trout tests we conducted concurrently for a separate project (63). Possible reasons for the infertility, which appeared to be random, include physical inability to spawn because the substrates were too small or behavioral changes caused by fish density or the nature of the heirarchal relationships. It is also unknown whether all trout from this stock reach reproductive maturity in 2 years.

In the bluegill test, the fish were probably too young to spawn extensively. Better results might be achieved by beginning the test with 2-yr-old specimens. Improved techniques for incubating embryos and rearing fry should be developed which include verified methods of disease control and proven diets. Until the fry can consume brine shrimp nauplii, it would be advisable to feed them laboratory cultures of rotifers, for example, instead of "green" water because the latter might contain parasites and pathogens. Also, maintenance of high food densities, such as cultures of rotifers in phytoplankton, might cause a significant proportion of the toxicant to be sorbed to or assimilated by the food. This could alter the mode of intoxication if not the toxicant concentration. Finally, some consideration should should be directed to the adequacy of the bluegill spawning substrates and the validity of spawn size estimates, for embryos are often spread about the adult tank, bound to debris, and eaten prior to being checked by the investigator. While the substrates were acceptable to the adults, it was difficult to remove the embryos with a fine brush. To our knowledge, no one has carefully evaluated different methods of embryo removal with reference to the injury they cause.

The conduct of chronic tests with fathead minnow is fairly routine with proper experience, and the only improvement which is recommended is to gain a better fix on the type and concentration of chemicals used for controlling fungus during embryo incubation.

In the test using cladocerans, it might be better to separately assess growth because there are not enough $f_0$-generation specimens available in the recommended procedure (60) and $f_1$-generation instars produced with a given week will of course differ in age.

In retrospect, *Hyallela azteca* and *Chironomus* No. 51 are not the most desirable species for chronic tests. Newly-hatched *H. azteca* are really too small for rapid enumeration or capture and would hamper a hatching success determination. A species such as *Gammarus lacustris* would be more desirable because it is larger (around 20 mm in adults) and quite widespread throughout the United States. *Chironomus* No. 51, in addition to the liability that it has not been taxonomically described, has the embryos helically arranged within the skein, which makes it very time-consuming to accurately count and separate embryos for a hatching success determination.

## ACCUMULATION OF CHLORDANE

Technical chlordane was accumulated extensively in *H. azteca* and *D. magna*, but not at all in the winged adults of *Chironomus* No. 51. For both amphipods and daphnids, tissue concentrations of a particular component were fairly proportional to aqueous concentration. However, both species tended to concentrate the various compounds to different extents. Concentration of the components was comparable between amphipods and daphnids, except that amphipods concentrated at least 2-times more of the chlordenes and heptachlor than daphnids.

The absence of notable residues of technical chlordane in adult chironomids exposed to both 0.7 and 1.0 µg/l chlordane in separate experiments is difficult to interpret. Recent studies of DDE uptake by fourth instar *C. tentans* by Derr and Zabik (68) indicate a passive mode of uptake and an essentially linear relationship between aqueous and tissue DDE concentration. Similar findings have been reported earlier by Kerr and Vass (69). Although *Chironomus* No. 51 may possess efficient mechanisms for metabolizing or excreting technical chlordane components at all developmental stages or during the transition from larva to adult, additional experiments designed to monitor the water-tissue concentration relationships for all developmental stages and several toxicant concentrations are needed to clarify the somewhat anomalous results we obtained for this species.

For both amphipods and daphnids, comparisons of the proportions of the chlordane components in the tissues with those in the neat material indicated that most were stored in different proportions. Cis- and trans-nonachlor were stored to a greater extent in both species than the other components. Similar findings have been made for fish collected from Lakes Superior and Huron (personal communication, L. Mueller, EPA, ERL-D). Since there appeared to be little if any compositional change relative to the neat material when technical chlordane was measured in aqueous solution, these components were either taken up preferentially, were metabolites of other constituents, or were particularly refractory to metabolism. The lower tissue ratio of heptachlor to cis- and trans-chlordane and that of the chlordane isomers to the nonachlor isomers suggests that these compounds may have been either taken up less efficiently by the two invertebrates, converted to nonachlors, or preferentially metabolized and excreted. The relationships may also be altered to some extent by the aqueous concentration since there were notable diminutions in the ratios of cis-/trans-nonachlor and to a much lesser extent for cis-/trans-chlordane at the highest insecticide levels. It can be speculated that if metabolic processes had a role in altering the proportions of the constituents in the tissues, uptake may have been sufficient in the brief period of exposure to temporarily supercede metabolic processes.

Obviously, these observations raise basic questions as to the relative uptake, metabolism, and excretion of these constituents, questions which can only be resolved by additional study. Such investigations are beneficial since the stored compounds may have different toxicological properties which would underlie any potential effects on predators. Laboratory studies of bioaccumulation should also be integrated with controlled experiments in semi-natural environments and with sampling of aquatic organisms in natural environments in order to verify the laboratory results.

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# APPENDIX TABLES

| No. | | Page |
|---|---|---|
| 1 | Approximate Composition of Technical Chlordane as Reflected in "Normalized" Chromatogram of Significant Peaks .................. | 92 |
| 2 | Representative Quality of Laboratory Water .................................................. | 93 |
| 3 | Standard Concentration-Percent Mortality Data Supplied by Committee on Methods For Toxicity Tests with Aquatic Organisms ........... | 94 |
| 4 | Comparison of Median Lethal Concentrations and 95% confidence Limits With Other Aquatic Toxicology Laboratories ..................... | 95 |
| 5 | Median Lethal Concentrations (LC50) for *Daphnia magna* and *Hyallela azteca* Exposed to Technical Chlordane ............................. | 96 |
| 6 | Median Lethal Concentrations (LC50) for Fathead Minnow Juveniles Exposed to Technical Chlordane ................................ | 97 |
| 7 | Median Lethal Concentrations (LC50) for Brook Trout Exposed to Technical Chlordane ........................................ | 98 |
| 8 | Median Lethal Concentrations (LC50) for Bluegill Exposed to Technical Chlordane ............. | 99 |
| 9 | Significance of Differences in 96-Hr LC50 Between *Daphnia magna*, Fathead Minnows, Brook Trout, and Bluegill .................. | 99 |
| 10 | Water Quality During Exposure of Fathead Minnows to Chlordane ........................ | 100 |

No. Page

11 Measured Technical Chlordane Concentrations During Chronic Toxicity Test Using Fathead Minnows ........ 104

12 Water Quality During Chronic Exposure of Bluegill to Technical Chlordane ........ 105

13 Technical Chlordane Concentrations During Chronic Toxicity Test Using Bluegill ........ 110

14 Water Quality During Chronic Toxicity Test of Technical Chlordane Utilizing Brook Trout ........ 111

15 Measured Concentrations of Technical Chlordane in Chronic Toxicity Test Utilizing Brook Trout ........ 116

16 Water Quality During Chronic Exposure of *Hyallela azteca* to Technical Chlordane ........ 118

17 Measured Concentrations of Technical Chlordane in Chronic Toxicity Test Utilizing *Hyallela azteca* ........ 119

18 Analysis of Variance of Wet Body Weights, Dry Body Weights and *Cis*-Chlordane Contents of *Hyallela azteca* Exposed to Different Concentrations of Technical Chlordane ........ 120

19 Water Quality During Chronic Exposure of *Daphnia magna* to Technical Chlordane ........ 121

20 Measured Concentrations of Technical Chlordane in Chronic Toxicity Test Using *Daphnia magna* ........ 122

21 Water Quality During Chronic Toxicity Tests Utilizing *Chironomus* No. 51 ........ 123

22 Measured Concentrations of Technical Chlordane in Chronic Toxicity Test Utilizing *Chironomus* No. 51 ........ 125

## APPENDIX TABLE 1. APPROXIMATE COMPOSITION OF TECHNICAL CHLORDANE AS REFLECTED IN "NORMALIZED" CHROMATOGRAM OF SIGNIFICANT PEAKS[a]

| Constituent | Percentage |
|---|---|
| $C_{10}H_7Cl_5$ - Diels-Alder Adduct (DAA): Pentachlorocyclopentadiene and cyclopentadiene ($C_5H_6$; "Cyclo") | 2 ± 1% |
| $C_{10}H_6Cl_6$ - Isomers in order of GLC retention time | |
| (1) Isomer-1, chlordane - DAA; hexachlorocyclopentadiene (Hex) and "Cyclo" | 1 ± 1% |
| (2) Isomer-2 | 7.5 ± 2% |
| (3) Isomers-3, 4 (combined) | 13 ± 2% |
| $C_{10}H_5Cl_7$ - Heptachlor | 10 ± 3% |
| $C_{10}H_6Cl_8$ - Chlordane isomers | |
| (1) *cis*-chlordane | 19 ± 3% |
| (2) *trans*-chlordane | 24 ± 2% |
| $C_{10}H_5Cl_9$ - Nonachlor | 7 ± 3% |
| Other constituents: | |
| Hex ($C_5Cl_6$) Maximum | 1% |
| Octachlorocyclopentene | 1 ± 1% |
| $C_{10}H_{7-8}Cl_{6-7}$ | 8.5 ± 2% |
| Constituents of lower GLC retention time than $C_5Cl_8$, including Hex | 2 ± 2% |
| Constituents of higher GLC retention time than nonachlor | 4 ± 3% |

[a] The foregoing approximations are based upon unadjusted values derived from moderate resolution gas-liquid chromatography. Apparent values obtained are typically influenced by conditions of analysis and the chromatographic systems employed, and the relative response sensitivity of the components. Under standardized conditions, these profiles are useful in comparing Technical Chlordane samples with Reference Technical Chlordane (taken from Velsicol Chemical Corp. [12]).

APPENDIX TABLE 2. REPRESENTATIVE QUALITY OF LABORATORY WATER

| Variable | Unit | Mean concentration | Variable | Unit | Mean concentration |
|---|---|---|---|---|---|
| Calcium | mg/l | 31.1 | Cyanide | mg/l | 0.0005 |
| Magnesium | mg/l | 13.1 | Iron | mg/l | 0.001 |
| Potassium | mg/l | 2.0 | Copper | mg/l | 0.005 |
| Sodium | mg/l | 15.4 | Zinc | mg/l | 0.001 |
| Chloride | mg/l | 11.3 | Cadmium | mg/l | 0.010 |
| Sulfate | mg/l | 8.6 | Chromium | mg/l | 0.025 |
| Sulfide | mg/l | <0.002 | pH |  | 7.70 |
| Nitrate | mg/l | 4.65 | Alkalinity | mg/l $CaCO_3$ | 166 |
| Nitrite | mg/l | 0.005 |  |  |  |
| Ammonia | mg/l $NH_3$-N | 0.16 | Acidity | mg/l $CaCO_3$ | 6 |
| Phenol | mg/l | 0.001 | Total hardness | mg/l $CaCO_3$ | 156 |
| Fluoride | mg/l | 0.96 | Specific conductance | µmhos/cm | 376 |

## APPENDIX TABLE 3. STANDARD CONCENTRATION-PERCENT MORTALITY DATA SUPPLIED BY COMMITTEE ON METHODS FOR TOXICITY TESTS WITH AQUATIC ORGANISMS[a]

| | Percent mortality | | | | | | |
|---|---|---|---|---|---|---|---|
| | Toxicant concentration, μg/l | | | | | | |
| Data set | Control | 7.8 | 13 | 22 | 36 | 60 | 100 |
| A | 0 | 0 | 0 | 10 | 100 | 100 | 100 |
| B | 0 | 0 | 0 | 70 | 100 | 100 | 100 |
| C | 0 | 0 | 0 | 10 | 40 | 100 | 100 |
| D | 0 | 0 | 0 | 20 | 70 | 100 | 100 |
| E | 0 | 0 | 0 | 20 | 30 | 100 | 100 |

[a] Data used to check the validity of statistical analyses of acute toxicity test results. Ten specimens/treatment.

APPENDIX TABLE 4. COMPARISON OF MEDIAN LETHAL CONCENTRATIONS AND 95% CONFIDENCE LIMITS WITH OTHER AQUATIC TOXICOLOGY LABORATORIES[a]

| | Our laboratory | | | | Average of eight other laboratories[b] | |
|---|---|---|---|---|---|---|
| | Litchfield and Wilcoxon (64) | | Computer program | | | |
| Data set | LC50, µg/l | 95% confidence limits for LC50 | LC50, µg/l | 95% confidence limits for LC50 | LC50,[c] µg/l | 95% confidence limits for LC50 |
| A | 25.4 | 22.1 - 29.2 | ...[d] | ... | 25.3<br>±1.9 | 20.4 - 32.0<br>±5.1 ±4.8 |
| B | 21.2 | 18.8 - 23.9 | ... | ... | 20.4<br>±0.9 | 14.8 - 49.3<br>±4.0 ±57.0 |
| C | 35.5 | 28.2 - 44.7 | 35.6 | 30.3 - 41.7 | 35.3<br>±2.4 | 27.5 - 45.1<br>±3.3 ±3.6 |
| D | 29.8 | 23.9 - 37.2 | 29.5 | 25.0 - 34.7 | 29.4<br>±0.8 | 23.5 - 36.9<br>±1.9 ±1.2 |
| E | 35.5 | 27.1 - 46.9 | 35.5 | 29.1 - 43.3 | 36.5<br>±3.4 | 28.2 - 46.8<br>±3.5 ±4.3 |

[a] All calculations performed on standard data given in Appendix Table 3.

[b] Results obtained through use of both manual and computer methods.

[c] Means ± 1 standard deviation are given for concentrations in µg/l.

[d] Calculations not made since computer was not programmed to process data having only one partial kill.

## APPENDIX TABLE 5. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR *DAPHNIA MAGNA* AND *HYALLELA AZTECA* EXPOSED TO TECHNICAL CHLORDANE

| Exposure time, hr | LC50, µg/l | 95% confidence limits for LC50, µg/l | $\hat{\sigma}$[a] | Log-probit regression equation[b] |
|---|---|---|---|---|
| *D. magna* - Test 1 | | | | |
| 70 | 31.1 | 27.1 - 35.7 | 0.1594 | $-4.37+6.28\ (\log x_i)$ |
| 96 | 28.4 | 25.3 - 31.9 | 0.1329 | $-5.94+7.53\ (\log x_i)$ |
| *D. magna* - Test 2 | | | | |
| 66[c] | 42.2 | 39.0 - 45.7 | 1.1121 | ... |
| 74[c] | 37.5 | 34.0 - 41.3 | 1.1726 | ... |
| 94[c] | 35.2 | 30.1 - 41.2 | 1.2910 | ... |
| *Hyallela azteca* | | | | |
| 168 | 97.1 | 70.9 - 133.0 | 0.4056 | $0.10+2.47\ (\log x_i)$ |

[a] Logarithm of the standard deviation of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b\ (\log x_i)$.

[c] Calculated according to the method of Litchfield and Wilcoxon (64). The slope function S replaces $\hat{\sigma}$, and is the antilogarithm of $\hat{\sigma}$.

### APPENDIX TABLE 6. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR FATHEAD MINNOW JUVENILES EXPOSED TO TECHNICAL CHLORDANE

| Exposure time, hr | LC50, µg/l | 95% confidence limits for LC50, µg/l | $\hat{\sigma}$[a] | Log-probit regression equation[b] |
|---|---|---|---|---|
| 45[c] | 53.4 | ... | ... | ... |
| 72 | 41.7 | 38.3 - 45.5 | 0.1022 | -10.86+9.79 (log $x_i$) |
| 96 | 36.9 | 33.0 - 41.3 | 0.1301 | - 7.27+7.69 (log $x_i$) |
| 120 | 35.9 | 32.6 - 39.5 | 0.112 | - 9.11+9.08 (log $x_i$) |
| 168 | 33.9 | 30.5 - 37.6 | 0.1175 | - 8.03+8.51 (log $x_i$) |
| 192 | 32.1 | 29.5 - 35.0 | 0.0956 | -10.76+10.46 (log $x_i$) |

[a] Logarithm of the standard deviation of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Median lethal time.

## APPENDIX TABLE 7. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR BROOK TROUT EXPOSED TO TECHNICAL CHLORDANE

| Exposure time, hr | LC50, µg/l | 95% confidence limits for LC50, µg/l | $\hat{\sigma}$[a] | Log-probit regression equation[b] |
|---|---|---|---|---|
| 27.2[c] | 125 | ... | ... | ... |
| 28.9[c] | 117 | ... | ... | ... |
| 46 | 102 | 93 - 112 | 0.0681 | $-24.49+14.68 (\log x_i)$ |
| 96[d] | 47 | ... | ... | ... |
| 118 | 39 | 34 - 44 | 0.0450 | $-30.30+22.21 (\log x_i)$ |
| 142 | 31 | 26 - 38 | 0.1494 | $15,05+6.69 (\log x_i)$ |
| 166 | 25 | 21 - 29 | 0.1192 | $18.49+8.39 (\log x_i)$ |

[a] Logarithm of the standard deviation of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b (\log x_i)$.

[c] Median lethal time.

[d] Interpolated from regression equation; (LC50) calculated from known LC50 values and exposure times.

## APPENDIX TABLE 8. MEDIAN LETHAL CONCENTRATIONS (LC50) FOR BLUEGILL EXPOSED TO TECHNICAL CHLORDANE

| Exposure time, hr | LC50, µg/l | 95% confidence limits for LC50, µg/l | $\hat{\sigma}$[a] | Log-probit regression equation[b] |
|---|---|---|---|---|
| 48 | 121 | 98 - 149 | 0.2051 | $-5.15+4.88\ (\log x_i)$ |
| 72 | 77 | 68 - 87 | 0.1397 | $-8.50+7.16\ (\log x_i)$ |
| 96 | 59 | 50 - 71 | 0.2057 | $-3.62+4.86\ (\log x_i)$ |
| 120 | 46 | 39 - 54 | 0.1759 | $-4.40+5.65\ (\log x_i)$ |
| 144 | 40 | 35 - 45 | 0.1233 | $-7.99+8.11\ (\log x_i)$ |

[a] Logarithm of the standard deviation of the population tolerance frequency distribution.

[b] Probit $\hat{y}_i = a + b\ (\log x_i)$.

## APPENDIX TABLE 9. SIGNIFICANCE OF DIFFERENCES IN 96-HR LC50 BETWEEN *DAPHNIA MAGNA*, FATHEAD MINNOWS, BROOK TROUT, AND BLUEGILL

| Comparison | t-value | Significance |
|---|---|---|
| *D. magna* vs fathead minnow | -1.754 | N.S. |
| Fathead minnow vs brook trout | -1.232 | N.S. |
| Brook trout vs bluegill | -1.094 | N.S. |
| *D. magna* vs brook trout | -3.520 | $p<0.001$ |
| *D. magna* vs bluegill | -2.989 | $p<0.001$ |
| Fathead minnow vs bluegill | -1.651 | N.S. |

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 12/11/72 | 21.5 ± 0.6 | 9.0 | 102.1 | 7.81 | 162 | 163 | ...[a] |
| 12/18/72 | 22.5 ± 0.9 | 8.2 | 93.0 | 7.80 | 158 | ... | ... |
| 12/25/72 | 23.8 ± 0.9 | 8.7 | 99.2 | 8.18 | 164 | 168 | ... |
| 1/1/73 | 23.2 ± 0.5 | 8.6 | 101.5 | 7.94 | 166 | 157 | ... |
| 1/8/73 | 24.0 ± 1.9 | 8.5 | 95.9 | 7.91 | 165 | 155 | ... |
| 1/15/73 | 24.1 ± 2.3 | 7.6 | 92.3 | 7.93 | 165 | 159 | ... |
| 1/22/73 | 23.4 ± 1.2 | 7.5 | 88.4 | 7.79 | 168 | 173 | ... |
| 1/29/73 | 24.7 ± 0.4 | 7.8 | 92.8 | 7.86 | 166 | 160 | ... |
| 2/5/73 | 24.5 ± 0.7 | 7.5 | 88.0 | 7.88 | 163 | 161 | ... |
| 2/12/73 | 24.9 ± 0.2 | 7.7 | 91.1 | 7.79 | 163 | 158 | ... |
| 2/19/73 | 25.0 ± 0.3 | 7.4 | 87.8 | 7.80 | 157 | 155 | ... |
| 2/26/73 | 25.0 ± 0.4 | ... | ... | 7.68 | 162 | 163 | 368 |
| 3/5/73 | 25.3 ± 0.2 | 7.4 | 88.6 | 7.67 | 168 | 172 | 337 |

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

| Date | Water temperature, ° C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 3/12/73 | 25.0 ± 0.3 | 6.6 | 77.5 | 7.70 | 163 | 158 | 360 |
| 3/19/73 | 24.8 ± 0.8 | 6.4 | 75.7 | 7.58 | 163 | 164 | 377 |
| 3/26/73 | 24.4 ± 1.4 | 6.8 | 80.4 | 7.60 | 163 | 167 | 327 |
| 4/2/73 | 25.1 ± 0.2 | 6.8 | 81.2 | 7.62 | 167 | 146 | 383 |
| 4/9/73 | 25.2 ± 0.3 | 6.9 | 81.9 | 7.50 | 161 | 142 | 360 |
| 4/16/73 | 25.3 ± 0.2 | 6.2 | 74.1 | 7.47 | 166 | 158 | 385 |
| 4/23/73 | 25.1 ± 0.7 | 6.4 | 77.8 | 7.50 | 160 | 148 | 380 |
| 4/30/73 | 25.3 ± 0.3 | 6.2 | 74.1 | 7.60 | 159 | 143 | 360 |
| 5/7/73 | 25.3 ± 0.2 | 6.8 | 81.4 | 7.70 | 162 | 145 | ... |
| 5/14/73 | 25.1 ± 0.4 | 5.7 | 68.1 | 7.45 | 166 | 161 | 410 |
| 5/21/73 | 24.9 ± 0.2 | 6.1 | 72.0 | 7.52 | 162 | 152 | 385 |
| 5/28/73 | 25.6 ± 0.1 | 6.0 | 72.8 | 7.73 | 160 | 160 | 400 |
| 6/4/73 | 25.3 ± 0.2 | 6.1 | 73.5 | 7.48 | 158 | 147 | 370 |

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 6/11/73 | 24.9 ± 0.1 | 5.9 | 82.0 | 7.55 | ... | 162 | 400 |
| 6/18/73 | 24.8 ± 0.1 | 6.0 | 71.1 | 7.73 | 169 | 153 | 388 |
| 6/25/73 | 24.8 ± 0.2 | 5.8 | 69.9 | 7.74 | 165 | 137 | 337 |
| 7/2/73 | 24.7 ± 0.4 | 6.5 | 73.0 | 7.60 | 169 | 152 | 351 |
| 7/9/73 | 24.4 ± 0.5 | 6.6 | 77.7 | 7.72 | 168 | 147 | 354 |
| 7/16/73 | 24.4 ± 0.3 | 6.4 | 75.7 | 7.64 | 172 | 154 | 375 |
| 7/23/73 | 24.9 ± 0.4 | 6.5 | 77.6 | 7.57 | 172 | 160 | 398 |
| 7/30/73 | 25.2 ± 0.1 | 6.6 | 78.1 | 7.62 | 170 | 152 | 379 |
| 8/6/73 | 24.9 ± 0.2 | 6.6 | 78.6 | 7.57 | 176 | 162 | 398 |
| 8/13/73 | 24.9 ± 0.1 | 6.6 | 79.9 | 7.65 | 174 | 159 | 408 |
| 8/20/73 | 24.9 ± 0.1 | 6.7 | 79.4 | 7.67 | 172 | 157 | 339 |
| 8/27/73 | 24.5 ± 0.1 | 7.3 | 86.9 | 7.73 | 169 | 155 | 376 |
| 9/3/73 | 24.3 ± 0.1 | 7.4 | 88.1 | 7.65 | 173 | 162 | 404 |

Continued ....

APPENDIX TABLE 10. WATER QUALITY DURING EXPOSURE OF FATHEAD MINNOWS TO CHLORDANE--continued

| Date | Water temperature, °C | Dissolved oxygen, |  | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
|  |  | mg/l | % saturation |  |  |  |  |
| 9/10/73 | 24.6 ± 0.3 | 6.9 | 87.3 | 7.67 | 168 | 160 | 375 |
| 9/24/73 | 24.3 ± 0.1 | 7.0 | 82.9 | 7.88 | 165 | 145 | 365 |
| 10/1/73 | 24.6 ± 0.3 | 6.9 | 82.2 | 7.70 | 170 | 166 | 405 |
| 10/8/73 | 24.3 ± 0.1 | ... | ... | 7.89 | 163 | 146 | 389 |
| Mean | ... | 6.9 | 82.5 | 7.70 | 166 | 156 | 376 |
| Standard deviation | ... | 0.8 | 8.7 | 0.15 | 5 | 8 | 23 |
| No. of observations[b] | ... | 237 | 237 | 180 | 180 | 178 | 61 |

[a] No observation. Majority of later conductance readings were composites collected from control, mid-range, and high chlordane concentrations.

[b] Monitored continuously.

### APPENDIX TABLE 11. MEASURED TECHNICAL CHLORDANE CONCENTRATIONS DURING CHRONIC TOXICITY TEST USING FATHEAD MINNOWS

| Sample date | Desired concentration of chlordane, μg/l | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.625 | 1.25 | 2.50 | 5.0 | 10.0 |
| 3/22/73 | 0.010 | 0.661 | 1.050 | 1.520 | 3.150 | 6.830 |
| 4/6/73 | 0.120 | 0.490 | 0.530 | 1.820 | 2.730 | 6.460 |
| 4/11/73 | 0.010 | 0.246 | 0.678 | 0.930 | 2.960 | 4.840 |
| 4/16/73 | 0.066 | 0.260 | 0.512 | 0.794 | 1.840 | 3.490 |
| 4/24/73 | Tr[a] | 0.341 | 0.646 | 1.720 | 3.280 | 6.970 |
| 4/30/73 | Tr | 0.238 | 0.585 | 0.860 | 1.750 | 3.450 |
| 5/7/73 | 0.082 | 0.190 | 0.678 | 0.214 | 0.358 | 0.888 |
| 5/18/73 | 0.039 | 0.397 | 0.755 | 1.530 | 3.520 | 5.790 |
| 5/24/73 | Tr | 0.226 | 0.684 | 1.290 | 3.460 | 7.070 |
| 5/30/73 | 0.140 | 0.552 | 0.716 | 1.660 | 3.590 | 2.920 |
| 6/8/73 | Tr | 0.362 | 0.728 | 2.340 | 3.950 | 7.710 |
| 6/13/73 | Tr | 0.338 | 0.429 | 1.240 | 2.380 | 4.500 |
| 6/20/73 | 0.080 | 0.198 | 1.500 | 0.264 | 0.322 | ...[b] |
| 7/3/73 | Tr | 0.444 | 0.906 | 1.750 | 3.300 | 7.420 |
| 7/11/73 | Tr | 0.557 | 0.836 | 1.910 | 3.830 | 6.380 |
| 7/18/73 | Tr | 0.526 | 0.960 | 1.800 | 3.880 | 7.690 |
| 7/26/73 | Tr | 0.514 | 0.854 | 1.590 | 3.060 | 7.000 |
| 8/16/73 | Tr | 0.072 | 0.437 | 1.510 | 2.620 | 6.440 |
| 8/30/73 | Tr | 0.136 | ...[c] | ...[c] | ...[c] | 5.430 |
| 9/6/73 | Tr | 0.246 | ... | ... | 2.840 | ...[b] |
| 9/13/73 | Tr | 0.311 | ... | ... | ... | ...[c] |
| 9/17/73 | Tr | 0.614 | ... | ... | ... | 8.580 |
| 9/27/73 | Tr | 0.437 | ... | ... | 4.360 | 11.420 |
| 10/4/73 | Tr | 0.290 | ... | ... | ... | ...[c] |
| 10/9/73 | Tr | ...[b] | ... | ... | 2.620 | 5.280 |
| Mean | Tr | 0.360 | 0.749 | 1.375 | 2.780 | 6.027 |
| Standard deviation | ... | 0.159 | 0.254 | 0.570 | 1.058 | 2.248 |
| No. of observations | 24 | 24 | 18 | 18 | 21 | 21 |

[a] Trace amounts of less than 5 ng/l chlordane detected.

[b] No observation.

[c] No measurements made because of no fish in test container.

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 12/12/72[a] | 26.5 ± 0.4[b] | 5.0 | 61.2 | 7.65 | 164 | 154 | ...[c] |
| 12/20/72 | 19.4 ± 0.5 | 6.2 | 67.7 | 7.46 | 155 | 173 | ... |
| 12/27/72 | 19.5 ± 0.7 | 5.5 | 59.9 | 7.54 | 167 | 171 | ... |
| 1/3/73 | 19.1 ± 0.5 | 6.5 | 70.2 | 7.58 | 164 | 157 | ... |
| 1/10/73 | 19.2 ± 0.9 | 5.6 | 61.1 | 7.55 | 170 | 157 | ... |
| 1/19/73 | 19.3 ± 0.5 | 6.3 | 60.4 | 7.60 | 166 | 157 | ... |
| 1/24/73 | 18.8 ± 0.6 | 5.6 | 60.4 | 7.50 | 166 | 161 | ... |
| 1/31/73 | 20.4 ± 0.1 | 5.9 | 63.0 | 7.53 | 165 | 159 | ... |
| 2/7/73 | 20.2 ± 0. | 4.9 | 53.2 | 7.48 | 165 | 164 | ... |
| 2/14/73 | 20.1 ± 0.1 | 5.5 | 60.0 | 7.54 | 157 | 156 | ... |
| 2/21/73 | 20.2 ± 0.1 | 5.6 | 52.6 | 7.49 | 167 | 167 | ... |
| 2/28/73 | 20.1 ± 0.1 | 5.2 | 56.7 | 7.40 | 160 | ... | ... |
| 3/7/73[d] | 20.6 ± 0. | 4.8 | 51.8 | 7.32 | 168 | 166 | 380 |

Continued ....

## APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

continued

| Date | Water temperature, °C | Dissolved oxygen, | | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| | | mg/l | % saturation | | | | |
| 3/14/73 | 20.4 ± 0.2 | 5.3 | 57.5 | 7.43 | 163 | 166 | 353 |
| 3/19/73 | 20.3 ± 0.2 | 5.0 | 54.7 | ... | ... | ... | ... |
| 3/21/73 | 20.1 ± 0.1 | 4.7 | 50.6 | 7.26 | 163 | 172 | 353 |
| 3/28/73 | 20.1 ± 0.1 | 4.3 | 46.9 | 7.32 | 164 | 171 | 377 |
| 3/31/73[e] | ... | 7.0 | 81.5 | ... | ... | ... | ... |
| 4/4/73 | 21.1 ± 1.2 | 6.3 | 68.6 | 7.37 | 168 | 155 | 380 |
| 4/11/73 | 23.9 ± 0.5 | 6.3 | 85.2 | 7.52 | 160 | 143 | 362 |
| 4/18/73 | 23.8 ± 1.6 | 5.8 | 67.8 | 7.50 | 166 | 158 | 360 |
| 4/25/73[f] | 25.0 ± 0.5 | 4.0 | 48.5 | ... | ... | ... | ... |
| 4/26/73[f] | ... | 4.6 | 54.4 | 7.32 | 161 | 146 | 370 |
| 4/30/73 | 26.7 ± 1.1 | 6.3 | 78.9 | ... | ... | ... | ... |
| 5/2/73 | ... | 5.5 | 69.2 | 7.62 | 163 | 159 | ... |
| 5/9/73 | 27.6 ± 0.3 | 5.8 | 69.6 | 7.73 | 162 | 146 | 393 |

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

continued

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 5/17/73 | 28.0 ± 0.2 | 6.0 | 76.0 | 7.73 | 164 | 155 | 420 |
| 5/23/73 | 28.1 ± 0.3 | 5.7 | 72.6 | 7.53 | 161 | 143 | 387 |
| 5/31/73 | 28.2 ± 0.2 | 6.5 | 81.6 | 7.91 | 155 | 155 | ... |
| 6/6/73 | 28.2 ± 0.3 | 5.1 | 64.7 | 7.54 | 160 | 146 | 385 |
| 6/13/73 | 28.0 ± 0.1 | 6.2 | 78.1 | 7.74 | 161 | 147 | 370 |
| 6/20/73 | 27.9 ± 0.1 | 5.9 | 74.1 | 7.82 | 164 | 153 | 390 |
| 6/27/73 | 28.0 ± 0.1 | 6.5 | 82.1 | 8.02 | 175 | 159 | 395 |
| 7/3/73 | 27.9 ± 0.2 | 5.8 | 73.8 | 7.73 | 167 | 145 | 375 |
| 7/10/73 | 27.8 ± 0.1 | 6.6 | 83.3 | 7.85 | 167 | 148 | 370 |
| 7/17/73 | 27.7 ± 0.1 | 6.1 | 76.6 | 7.78 | 166 | 141 | 375 |
| 7/24/73 | 27.9 ± 0.1 | 6.7 | 85.0 | 7.94 | 167 | 146 | 380 |
| 7/31/73 | 28.0 ± 0.1 | 6.4 | 80.5 | 7.70 | 170 | 152 | 400 |
| 8/6/73 | 27.7 ± 0.1 | 6.8 | 84.0 | 7.84 | 173 | 153 | 390 |

Continued ....

APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE
continued

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| 8/13/73 | 27.9 ± 0.2 | 6.1 | 77.2 | 7.74 | 173 | 159 | 395 |
| 8/20/73 | 28.0 ± 0.1 | 5.6 | 70.9 | 7.71 | 173 | 157 | 385 |
| 8/27/73 | 27.7 ± 0.1 | 6.1 | 77.2 | 7.69 | 169 | 154 | 385 |
| 9/4/73 | 27.7 ± 0.1 | 6.4 | 80.6 | 7.79 | 173 | 159 | 394 |
| 9/11/73 | 27.9 ± 0.2 | 5.8 | 69.1 | 7.72 | 169 | 158 | 371 |
| 9/18/73 | 27.8 ± 0.1 | ... | ... | ... | ... | ... | ... |
| 9/25/73 | 27.4 ± 0.2 | 6.6 | 82.8 | 7.91 | 165 | 144 | 368 |
| 10/2/73[g] | 27.6 ± 0.2 | 7.0 | 88.3 | 7.93 | 169 | 162 | 390 |
| 10/9/73 | 27.4 ± 0.1 | 6.9 | 87.5 | 7.98 | 161 | 145 | 386 |
| 10/16/73 | 27.9 ± 0.1 | 6.3 | 80.1 | 7.80 | 166 | 157 | 386 |
| 11/23/73 | 27.9 ± 0.1 | 6.9 | 87.4 | 7.92 | 159 | 146 | 368 |

Continued ....

## APPENDIX TABLE 12. WATER QUALITY DURING CHRONIC EXPOSURE OF BLUEGILL TO TECHNICAL CHLORDANE

continued

| Date | Water temperature, °C | Dissolved oxygen, mg/l | Dissolved oxygen, % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|
| Mean | 24.7 ± 3.8 | 5.9 | 69.9 | 7.65 | 165 | 156 | 380 |
| Standard deviation | ... | 0.7 | 11.8 | 0.20 | 5 | 9 | 14 |
| No. of observations | 267 | 279 | 279 | 184 | 184 | 184 | 31 |

[a] Dissolved oxygen determined with Yellow Springs Instrument Company oxygen meter (Model No. 54).

[b] Values given are means, except for water temperature where mean ± 1 standard deviation is given.

[c] No observation.

[d] Dissolved oxygen determined with azide modification of Winker method (21).

[e] Artificial aeration instituted.

[f] Malfunction of aeration equipment.

[g] Mean acidity from 10/2/73 to 11/23/73 was 4.42 mg/l $CaCO_3$ with a standard deviation of 2.44.

## APPENDIX TABLE 13. TECHNICAL CHLORDANE CONCENTRATIONS DURING CHRONIC TOXICITY TEST USING BLUEGILL

| Sample date | Nominal concentration of chlordane, μg/l | | | | | |
|---|---|---|---|---|---|---|
| | Control | 0.625 | 1.25 | 2.5 | 5 | 10 |
| 3/26/73[a] | 0.08 | 0.06 | 0.93 | 0.72 | 1.95 | 2.52 |
| 3/20/73 | 0.06 | 0.26 | 0.87 | 0.94 | 2.10 | 5.84 |
| 4/5/73 | 0.05 | 0.05 | 0.22 | 0.36 | 1.00 | 2.58 |
| 4/10/73 | 0.00 | 0.25 | 0.23 | 1.07 | 2.86 | 3.75 |
| 4/16/73 | 0.07 | 0.26 | 0.46 | 1.02 | 2.34 | 4.16 |
| 4/25/73 | 0.01 | 0.19 | 0.46 | 1.16 | 2.38 | 5.24 |
| 4/30/73 | 0.00 | 0.24 | 0.26 | 0.85 | 1.43 | 3.56 |
| 5/7/73 | 0.00 | 0.29 | 0.53 | 1.98 | 2.12 | 4.76 |
| 5/18/73 | 0.09 | 0.30 | 0.61 | 1.70 | 2.71 | 5.98 |
| 5/29/73 | 0.02 | 0.28 | 0.53 | 1.34 | 2.51 | 6.32 |
| 6/4/73 | 0.02 | 0.38 | 0.34 | 0.80 | 1.98 | 4.49 |
| 6/6/73 | 0.00 | 0.00 | 0.17 | 1.16 | 1.95 | 4.86 |
| 6/13/73 | 0.00 | 0.17 | 0.56 | 1.30 | 2.81 | 6.64 |
| 6/19/73 | 0.00 | 0.34 | 0.52 | 1.29 | 2.44 | 6.69 |
| 7/2/73 | 0.00 | 0.32 | 0.73 | 1.23 | 2.27 | 4.83 |
| 7/11/73 | 0.00 | 0.33 | 0.74 | 1.43 | 2.78 | 7.42 |
| 7/18/73 | 0.00 | ...[b] | ...[b] | 0.39 | ...[b] | ...[b] |
| 7/27/73 | 0.00 | 0.33 | 0.78 | 1.43 | 2.90 | 7.77 |
| 8/1/73 | 0.00 | ... | 0.35 | 1.22 | 2.19 | 4.37 |
| 8/17/73 | 0.00 | 0.11 | 0.43 | 1.55 | 2.00 | 5.36 |
| 8/30/73 | 0.00 | 0.19 | ...[b] | 0.66 | 0.65 | 3.44 |
| 9/6/73 | 0.00 | 0.21 | 0.48 | 0.42 | 1.73 | 4.18 |
| 9/14/73 | 0.00 | 0.51 | 0.64 | 1.20 | 2.28 | 8.88 |
| 9/21/73 | 0.00 | 0.31 | 0.42 | 1.25 | 2.88 | 6.15 |
| 9/27/73 | 0.00 | 0.37 | 0.72 | 2.90 | ...[b] | ...[b] |
| 10/4/73 | 0.00 | 0.44 | 0.83 | 1.85 | 2.36 | 5.32 |
| 10/9/73 | 0.00 | 0.15 | 0.57 | 1.49 | ...[b] | ...[b] |
| 10/19/73 | 0.00 | 0.26 | 0.60 | 1.47 | 2.48 | 4.11 |
| Mean | 0.01 | 0.25 | 0.54 | 1.22 | 2.20 | 5.17 |
| Standard deviation | 0.03 | 0.12 | 0.21 | 0.53 | 0.56 | 1.57 |

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[a] Because of analytical problems, data prior to this date were not considered reliable, and consequently not entered.

[b] Other hexane-soluble substances encountered in water samples which interferred with chlordane measurement.

## APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT

| Date | Water temperature, °C | Dissolved oxygen mg/l | Dissolved oxygen % saturation | pH | Total alkalinity, mg/l CaCO$_3$ | Acidity, mg/l CaCO$_3$ | Total hardness, mg/l CaCO$_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| 3/30/73[a] | 10.8 ± 0.5[b] | 7.6 | 68.3 | 7.37 | 164 | ... | 143 | ... |
| 4/3/73 | 11.6 ± 0.5 | 7.6 | 68.5 | 7.10 | 163 | ... | 164 | 370 |
| 4/10/73 | 10.2 ± 1.5 | 7.8 | 67.1 | 7.10 | 162 | ... | 154 | 375 |
| 4/16/73 | 11.3 ± 1.5 | 7.9 | 69.9 | 7.25 | 164 | ... | 157 | 368 |
| 4/23/73 | 12.1 ± 0.8 | 8.7 | 83.4 | 7.50 | 160 | ... | 148 | 370 |
| 4/30/73 | 12.9 ± 0.7 | 8.9 | 82.5 | 7.48 | 165 | ... | 158 | 370 |
| 5/8/73 | 12.8 ± 0.3 | 9.0 | 85.1 | 7.60 | 162 | ... | 148 | 400 |
| 5/14/73 | 13.2 ± 0.5 | 8.9 | 84.2 | 7.40 | 164 | ... | 160 | 395 |
| 5/21/73 | 14.1 ± 0.6 | 7.0 | 66.8 | 7.37 | 162 | ... | 149 | 390 |
| 5/29/73 | 14.3 ± 0.2 | 6.3 | 60.9 | 7.69 | 159 | ... | 160 | 405 |
| 6/5/73 | 14.8 ± 0.3 | 6.4 | 62.3 | 7.32 | 158 | ... | 148 | 375 |
| 6/11/73[c] | 14.8 ± 0.1 | 8.0 | 78.6 | 7.52 | ... | ... | 160 | 390 |
| 6/18/73[d] | 14.6 ± 0.2 | 7.2 | 70.2 | 7.61 | 166 | ... | 151 | 385 |
| 6/25/73 | 14.2 ± 0.1 | 6.9 | 67.3 | 7.71 | 164 | ... | 141 | 360 |

Continued

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

| Date | Water temperature, °C | Dissolved oxygen |  | pH | Total alkalinity, | Acidity, | Total hardness, | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
|  |  | mg/l | % saturation |  | mg/l $CaCO_3$ |  |  |  |
| 7/2/73 | 14.0 ± 0.2 | 7.5 | 73.3 | 7.47 | 168 | ... | 153 | 380 |
| 7/9/73 | 14.5 ± 0.4 | 7.4 | 71.4 | 7.50 | 170 | ... | 154 | 375 |
| 7/16/73 | 14.4 ± 0.1 | 8.0 | 77.1 | 7.68 | 171 | ... | 154 | 385 |
| 7/24/73 | 14.3 ± 0.6 | 8.1 | 79.6 | 7.71 | 168 | ... | 148 | 380 |
| 7/30/73 | 14.5 ± 0.8 | 8.0 | 75.8 | 7.65 | 170 | ... | 152 | 390 |
| 8/6/73 | 14.4 ± 0.1 | 8.5 | 82.5 | 7.66 | 175 | ... | 162 | 400 |
| 8/15/73 | 14.8 ± 0.1 | 8.3 | 81.7 | 7.70 | 165 | ... | 145 | 385 |
| 8/21/73 | 15.1 ± 0.7 | 8.0 | 78.8 | 7.71 | 172 | ... | 158 | 390 |
| 8/27/73 | 15.0 ± 0.4 | 8.7 | 85.7 | 7.70 | 171 | ... | 156 | 380 |
| 9/4/73 | 13.7 ± 1.0 | 8.1 | 77.6 | 7.64 | 173 | ... | 162 | 402 |
| 9/10/73 | 13.1 ± 0.3 | 8.5 | 79.5 | 7.73 | 167 | ... | 156 | 373 |
| 9/18/73 | 11.8 ± 0.4 | 9.0 | 82.9 | 7.69 | 165 | 11.0 | 150 | 379 |
| 9/25/73 | 12.0 ± 0.3 | 8.4 | 78.2 | 7.84 | 166 | 6.8 | 144 | 372 |

Continued ....

## APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

| Date | Water temperature, °C | Dissolved oxygen |  | pH | Total alkalinity, | Acidity, | Total hardness, | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
|  |  | mg/l | % saturation |  | mg/l $CaCO_3$ |  |  |  |
| 10/3/73 | 10.8 ± 0.7 | 9.5 | 83.8 | 7.87 | 168 | ... | 164 | 405 |
| 10/11/73 | 12.0 ± 2.6 | 9.0 | 83.3 | 7.70 | 159 | 10.3 | 143 | 386 |
| 10/18/73 | 10.4 ± 0.6 | 9.4 | 83.6 | 7.63 | 165 | 6.8 | 160 | 397 |
| 10/23/73 | 10.6 ± 0.3 | 9.0 | 81.5 | 7.70 | 166 | 6.3 | 157 | 396 |
| 10/30/73 | 9.4 ± 1.3 | 9.5 | 82.2 | 7.76 | 159 | 4.5 | 145 | 379 |
| 11/5/73 | 10.0 ± 0.8 | 10.0 | 87.7 | 7.80 | 163 | 6.0 | 152 | 387 |
| 11/12/73 | 10.2 ± 0.4 | 8.8 | 78.9 | 7.88 | 166 | 11.1 | 159 | 411 |
| 11/19/73 | 10.2 ± 0.5 | 9.2 | 82.2 | 7.66 | 143 | 4.5 | 140 | ... |
| 11/26/73 | 10.7 ± 1.3 | 8.6 | 75.2 | 7.82 | 145 | 14.3 | 141 | 374 |
| 12/3/73 | 10.2 ± 0.3 | 9.8 | 87.1 | 8.15 | 153 | 4.4 | 160 | ... |
| 12/10/73 | 10.2 ± 0.2 | 9.6 | 85.7 | 7.82 | 143 | 8.0 | 143 | 377 |
| 12/17/73 | 10.1 ± 0.1 | 9.6 | 85.0 | 8.04 | 145 | 7.1 | 133 | 357 |
| 12/24/73 | 10.2 ± 0.2 | 9.9 | 87.8 | 8.53 | 158 | ... | 139 | 375 |

Continued ....

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT --continued

| Date | Water temperature, ℃ | Dissolved oxygen mg/l | Dissolved oxygen % saturation | pH | Total alkalinity, mg/l CaCO$_3$ | Acidity, mg/l CaCO$_3$ | Total hardness, mg/l CaCO$_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| 12/31/73 | 9.6 ± 0.4 | 9.4 | 83.0 | 7.83 | 155 | 11.8 | 142 | 394 |
| 1/7/74 | 9.7 ± 0.2 | 10.1 | 88.7 | 8.00 | 158 | 11.5 | 144 | 387 |
| 1/14/74 | 9.4 ± 0.4 | 10.0 | 86.2 | 7.96 | 146 | 8.6 | 135 | 365 |
| 1/21/74 | 9.6 ± 0.5 | 9.4 | 81.7 | 7.83 | 140 | 7.7 | 136 | 376 |
| 1/28/74 | 9.8 ± 0.6 | 9.7 | 84.4 | 7.82 | 144 | 6.3 | 145 | 380 |
| 2/4/74 | 8.9 ± 0.3 | 9.6 | 84.7 | 7.87 | 155 | 10.7 | 142 | 369 |
| 2/11/74 | 9.2 ± 0.4 | 9.8 | 86.7 | 7.83 | 155 | 6.3 | 136 | 367 |
| 2/18/74 | 9.7 ± 0.4 | 9.5 | 84.5 | 7.79 | 156 | 5.8 | 148 | 380 |
| 2/25/74 | 10.3 ± 0.2 | 8.5 | 76.2 | 7.86 | 151 | 6.2 | 139 | 361 |
| 3/4/74 | 10.1 ± 0.4 | 9.6 | 85.4 | 7.92 | 153 | 4.2 | 142 | 367 |
| 3/11/74 | 10.5 ± 0.2 | 8.2 | 72.9 | 7.83 | 158 | 6.0 | 147 | 374 |
| 3/18/74 | 10.3 ± 0.2 | 6.8 | 59.7 | 7.87 | 158 | 4.8 | 135 | 356 |
| 3/25/74 | 10.2 ± 0.2 | 6.8 | 60.1 | 7.73 | 156 | 4.3 | 136 | 376 |

Continued ....

APPENDIX TABLE 14. WATER QUALITY DURING CHRONIC TOXICITY TEST OF TECHNICAL CHLORDANE UTILIZING BROOK TROUT--continued

| Date | Water temperature, °C | Dissolved oxygen mg/l | Dissolved oxygen % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Acidity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| 4/1/74 | 10.0 ± 0.3 | 6.6 | 59.1 | 7.82 | 149 | 3.5 | 134 | 358 |
| 4/8/74 | 9.9 ± 0.1 | 7.3 | 71.1 | 7.77 | 163 | 5.4 | 162 | 421 |
| 4/15/74 | 10.2 ± 0.4 | 7.1 | 63.3 | 7.62 | 160 | 7.3 | 160 | 413 |
| 4/22/74 | 10.8 ± 0.6 | 6.7 | 62.9 | 7.62 | 160 | 6.8 | 157 | 417 |
| Mean | ... | 8.3 | 75.7 | 7.68 | 160 | 7.3 | 150 | 380 |
| Standard deviation | ... | 1.2 | 9.9 | 0.26 | 8 | 2.7 | 10 | 23 |
| No. of observations | 419 | 247 | 247 | 174 | 174 | 79 | 174 | 73 |

[a] Dissolved oxygen determined with azide modification of Winkler method (21).

[b] Values are means. Standard deviations given for temperature data.

[c] Artificial aeration instituted.

[d] Dissolved oxygen determined with Yellow Springs Instrument Company Oxygen Meter after this date.

## APPENDIX TABLE 15. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING BROOK TROUT

|  | Nominal chlordane concentration, μg/l[a] |  |  |  |  |
|---|---|---|---|---|---|
| Date | 0.625 | 1.25 | 2.5 | 5.0 | 10.0 |
| 4/9/73 | 0.43 | 0.77 | 1.84 | 2.84 | 7.14 |
| 4/11/73 | 0.42 | 0.64 | 1.84 | 3.18 | 6.44 |
| 4/16/73 | 0.34 | 0.92 | 2.36 | 4.01 | 7.93 |
| 4/24/73 | 0.35 | 0.49 | 1.68 | 1.99 | 6.80 |
| 5/1/73 | 0.03 | 0.06 | 0.16 | 0.23 | 0.41 |
| 5/7/73 | 0.36 | 0.76 | 1.27 | 2.28 | 5.79 |
| 5/18/73 | 0.34 | 0.66 | 1.32 | 1.97 | 7.07 |
| 5/24/73 | 0.28 | 0.59 | 1.34 | 2.14 | 8.42 |
| 6/4/73 | 0.22 | 0.97 | 1.72 | 3.28 | 2.02 |
| 6/6/73 | 0.22 | 0.55 | 1.57 | 2.48 | 7.86 |
| 6/13/73 | 0.24 | 0.66 | 1.59 | 3.44 | 6.29 |
| 6/19/73 | 0.42 | 0.57 | 1.28 | 2.05 | 9.24 |
| 7/3/73 | ...[b] | 1.28 | 1.49 | 3.14 | ... |
| 7/11/73 | 0.31 | 1.24 | 1.14 | 1.57 | 9.71 |
| 7/18/73 | 0.18 | 0.87 | ... | 1.39 | 6.45 |
| 7/26/73 | 0.33 | 0.63 | 1.14 | 3.54 | 8.43 |
| 8/1/73 | 0.10 | 0.92 | 1.22 | 3.11 | 4.02 |
| 8/16/73 | 0.09 | 0.17 | 0.50 | 1.44 | 6.46 |
| 8/30/73 | 0.23 | 0.67 | 0.96 | 1.28 | 6.58 |
| 9/6/73 | 0.14 | 0.49 | 0.90 | 2.78 | 7.25 |
| 9/13/73 | 0.41 | 0.35 | 0.36 | 0.90 | 3.49 |
| 9/21/73 | 0.24 | 0.42 | 0.72 | 1.48 | 6.36 |
| 9/27/73 | 0.31 | ... | 1.55 | 3.96 | ... |
| 10/4/73 | 0.37 | 0.49 | 1.11 | 1.25 | 5.30 |
| 10/9/73 | 0.41 | 0.43 | 1.23 | 1.11 | 6.79 |
| 10/19/73 | 0.25 | 0.30 | 0.90 | 0.15 | 0.37 |
| 10/26/73 | 0.37 | 0.45 | 0.84 | 1.86 | 8.01 |
| 11/16/73 | 0.45 | 0.94 | 1.39 | 2.24 | 3.70 |
| 11/23/73 | 0.28 | 0.65 | 1.58 | 2.35 | 5.35 |
| 11/30/73 | 0.32 | 0.76 | 1.27 | 1.82 | 6.97 |
| 12/14/73 | 0.21 | 0.56 | 0.96 | 1.83 | ... |
| 12/21/73 | 0.20 | 0.31 | 0.59 | 1.03 | 3.18 |
| 12/28/73 | 0.25 | 0.48 | 0.99 | 1.75 | 4.53 |
| 1/2/74 | 0.28 | 0.55 | 0.90 | 1.72 | 7.63 |
| 1/8/74 | 0.40 | 0.63 | 1.18 | 2.14 | 5.74 |
| 1/15/74 | 0.30 | 0.63 | 1.34 | 2.42 | 5.24 |
| 1/25/74 | 0.32 | 0.69 | 0.91 | 2.71 | 4.98 |

Continued ....

## APPENDIX TABLE 15. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING BROOK TROUT--continued

| | Nominal chlordane concentration, μg/l[a] | | | | |
|---|---|---|---|---|---|
| Date | 0.625 | 1.25 | 2.5 | 5.0 | 10.0 |
| 2/5/74 | 0.39 | 0.74 | 1.52 | 1.98 | ... |
| 2/14/74 | 0.10 | 0.84 | 1.10 | 2.34 | 4.31 |
| 2/21/74 | 0.42 | 0.69 | 1.39 | 2.45 | ... |
| 2/27/74 | 0.65 | 0.73 | 1.23 | 2.10 | 4.79 |
| 3/6/74 | ... | ... | 1.95 | 2.56 | ... |
| 3/13/74 | 0.30 | 0.52 | 0.91 | 1.42 | 2.71 |
| 3/20/74 | 1.16 | 1.16 | 2.62 | 2.88 | ... |
| 3/28/74 | 0.22 | 0.40 | 0.90 | 1.64 | 3.38 |
| 4/10/74 | 0.29 | 0.74 | 1.58 | 2.23 | 4.74 |
| 4/16/74 | 0.41 | 0.79 | 1.70 | 2.80 | 6.29 |
| 4/23/74 | 0.37 | 0.78 | 1.61 | 2.52 | 4.51 |
| 5/2/74 | 0.65 | 1.11 | 2.06 | 4.37 | 7.86 |
| Mean | 0.32 | 0.66 | 1.29 | 2.21 | 5.80 |
| Standard deviation | 0.18 | 0.25 | 0.48 | 0.89 | 2.15 |
| No. of observations | 47 | 47 | 48 | 49 | 42 |

[a] Less than 0.11 μg/l chlordane measured in control at any time during test.

[b] No observation.

APPENDIX TABLE 16. WATER QUALITY DURING CHRONIC EXPOSURE OF HYALLELA AZTECA TO TECHNICAL CHLORDANE

| Date | Water temperature, °C | Dissolved oxygen mg/l | Dissolved oxygen % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Acidity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, μmhos/cm |
|---|---|---|---|---|---|---|---|---|
| 4/2/74 | 15.4 ± 0.6 | 7.6 | 74.8 | 7.97 | 151 | 3.7 | 135 | 359 |
| 4/10/74 | 15.3 ± 1.4 | 8.0 | 82.8 | 8.03 | 154 | 3.5 | 142 | 376 |
| 4/17/74 | 17.5 ± 0.3 | 7.7 | 77.8 | 7.63 | 162 | 7.7 | 161 | 419 |
| 4/24/74 | 17.5 ± 0.3 | 7.7 | 78.5 | 7.86 | 151 | 5.1 | 149 | 393 |
| 4/30/74 | 17.5 ± 1.4 | 7.6 | 78.7 | 7.80 | 146 | 6.9 | 151 | 385 |
| 5/6/74 | 16.5 ± 0.4 | 7.3 | 73.3 | 7.90 | 150 | 4.3 | 148 | 385 |
| 5/13/74 | 16.9 ± 0.1 | 7.3 | 72.7 | 7.83 | 152 | 5.5 | 150 | 401 |
| 5/20/74 | 16.7 ± 0.2 | 6.9 | 69.7 | 7.80 | 152 | 5.5 | 150 | 395 |
| Mean | 16.7 | 7.5 | 76.1 | 7.86 | 152 | 5.3 | 148 | 388 |
| Standard deviation | ±1.0 | ±0.4 | ±4.3 | ±0.13 | ±5 | ±1.5 | ±7 | ±18 |
| No. of measurements | 40 | 24 | 24 | 24 | 24 | 24 | 24 | 8 |

## APPENDIX TABLE 17. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING HYLALLELA AZTECA

| | Nominal chlordane concentration, µg/l[a] | | | | |
|---|---|---|---|---|---|
| Date | 2.5 | 5 | 10 | 20 | 40 |
| 3/28/74 | 0.24 | 0.72 | 1.08 | 3.32 | 5.77 |
| 4/5/74 | 0.52 | 0.83 | 1.72 | 3.06 | 6.46 |
| 4/10/74 | 0.82 | 1.69 | 3.71 | 6.47 | 13.99 |
| 4/16/74 | 1.93 | 4.25 | 3.16 | 14.50 | 19.20 |
| 4/23/74 | 1.82 | 3.31 | 9.65 | 16.40 | 33.50 |
| 5/3/74 | 1.28 | 2.91 | 5.05 | 13.70 | 26.40 |
| 5/7/74 | 2.68 | 4.31 | 9.92 | 21.40 | 28.80 |
| 5/17/74 | 1.85 | 2.95 | 7.57 | 12.60 | 25.45 |
| 5/23/74 | 1.56 | 2.75 | 6.00 | 12.30 | 25.10 |
| Mean | 1.41 | 2.64 | 5.32 | 11.53 | 20.52 |
| Standard deviation | 0.77 | 1.32 | 3.24 | 6.14 | 9.85 |

[a] No chlordane detected in control chambers, except on 4/10/74, when 0.21 µg/l was measured.

## APPENDIX TABLE 18. ANALYSIS OF VARIANCE OF WET BODY WEIGHTS, DRY BODY WEIGHTS AND *CIS*-CHLORDANE CONTENTS OF *HYALLELA AZTECA* EXPOSED TO DIFFERENT CONCENTRATIONS OF TECHNICAL CHLORDANE

| Source of error | Degrees of freedom | Sum of squares | Mean square | F |
|---|---|---|---|---|
| Wet body weights - replicate I | | | | |
| Among treatments | 4 | 39.62 | 9.90 | 6.71[a] |
| Within treatments | 95 | 140.13 | 1.47 | |
| Total | 99 | 179.75 | | |
| Wet body weights - replicate II | | | | |
| Among treatments | 4 | 61.55 | 15.38 | 7.39[a] |
| Within treatments | 100 | 201.54 | 2.01 | |
| Total | 104 | 263.09 | | |
| Dry body weights - both replicates | | | | |
| Among treatments | 4 | 0.47 | 0.11 | 3.53[b] |
| Within treatments | 5 | 0.16 | 0.03 | |
| Total | 9 | 0.63 | | |

[a] Significant at $p < 0.005$.

[b] Significant at $p < 0.001$.

## APPENDIX TABLE 19. WATER QUALITY DURING CHRONIC EXPOSURE OF *DAPHNIA MAGNA* TO TECHNICAL CHLORDANE

| Date | Water temperature, °C | Dissolved oxygen |  | pH | Total alkalinity, | Acidity, | Total hardness, | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
|  |  | mg/l | % saturation |  | mg/l $CaCO_3$ |  |  |  |
| 5/6/74 | 20.9 | 5.8 | 64.7 | 8.03 | 160 | 3.3 | 162 | 416 |
| 5/13/74 | 20.3 | 6.0 | 66.0 | 8.03 | 162 | 4.2 | 167 | 440 |
| 5/20/74 | 21.0 | 5.0 | 55.7 | 8.00 | 165 | 4.2 | 169 | 440 |
| 5/28/74 | 21.4 | ... | ... | 8.10 | 160 | 2.4 | 161 | 413 |
| Mean | 20.9 | 5.6 | 62.0 | 8.03 | 162 | 3.5 | 165 | 428 |
| Standard deviation | 0.5 | 0.5 | 5.1 | 0.06 | 3 | 0.8 | 4 | 15 |
| No. of observations | 4 | 9 | 9 | 12 | 12 | 12 | 12 | 4 |

## APPENDIX TABLE 20. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST USING *DAPHNIA MAGNA*

| | Nominal concentration of technical chlordane, µg/l | | | | | |
|---|---|---|---|---|---|---|
| Date | Control | 6.1 | 12.1 | 24.2 | 48.5 | 96.9 |
| 5/7/74 | 0[a] | 1.7 | 1.5 | 5.5 | 8.1 | 13.5 |
| 5/17/74 | 0 | 1.7 | 3.1 | 5.9 | 12.1 | 19.0 |
| 5/23/74 | 0 | 1.8 | 2.8 | 7.3 | 15.0 | 32.2 |
| Mean | 0 | 1.7 | 2.5 | 6.2 | 12.1 | 21.6 |
| Standard deviation | 0 | 0.1 | 0.9 | 1.0 | 3.6 | 9.6 |

[a] No chlordane detected.

## APPENDIX TABLE 21. WATER QUALITY DURING CHRONIC TOXICITY TESTS UTILIZING CHIRONOMUS NO. 51

| Date | Water temperature, °C | Dissolved oxygen mg/l | Dissolved oxygen % saturation | pH | Total alkalinity, mg/l $CaCO_3$ | Acidity, mg/l $CaCO_3$ | Total hardness, mg/l $CaCO_3$ | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
| **Test 1** | | | | | | | | |
| 5/6/74 | 24.0 | 6.8 | 80.3 | 7.93 | 155 | 4.0 | 154 | 393 |
| 5/13/74 | 24.0 | 7.1 | 82.3 | 7.97 | 154 | 4.7 | 154 | 406 |
| 5/20/74 | 23.6 | 6.3 | 75.0 | 7.93 | 153 | 4.5 | 153 | 401 |
| 5/28/74 | 23.1 | ...[a] | ... | 7.83 | 156 | 3.3 | 155 | 392 |
| Mean | 23.8 | 6.7 | 79.2 | 7.94 | 154 | 4.2 | 154 | 398 |
| Standard deviation | 0.3 | 0.4 | 3.6 | 0.05 | 2 | 0.7 | 2 | 7 |
| No. of observations | 9 | 9 | 9 | 12 | 12 | 12 | 11 | 4 |
| **Test 2** | | | | | | | | |
| 6/6/74 | 24.7 | 7.1 | 84.3 | 7.90 | 158 | 4.5 | 155 | 398 |
| 6/11/74 | 24.6 | 7.0 | 82.2 | 7.99 | 147 | 2.8 | 141 | 351 |

Continued

## APPENDIX TABLE 21. WATER QUALITY DURING CHRONIC TOXICITY TESTS UTILIZING CHIRONOMUS NO. 51--continued

| Date | Water temperature, °C | Dissolved oxygen |  | pH | Total alkalinity, | Acidity, | Total hardness, | Specific conductance, µmhos/cm |
|---|---|---|---|---|---|---|---|---|
|  |  | mg/l | % saturation |  | mg/l $CaCO_3$ |  |  |  |
| 6/21/74 | 24.4 | 6.9 | 80.3 | 7.77 | 160 | 5.4 | 160 | 394 |
| 6/24/74 | 24.2 | ... | ... | 7.87 | 155 | 4.8 | 151 | 380 |
| Mean | 24.4 | 7.0 | 82.3 | 7.90 | 153 | 4.2 | 150 | 380 |
| Standard deviation | 0.3 | 0.1 | 2.0 | 0.10 | 5 | 1.1 | 8 | 21 |
| No. of observations | 22 | 9 | 9 | 18 | 18 | 18 | 18 | 4 |

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[a] No observation.

## APPENDIX TABLE 22. MEASURED CONCENTRATIONS OF TECHNICAL CHLORDANE IN CHRONIC TOXICITY TEST UTILIZING *CHIRONOMUS* NO. 51

| Date | Measured chlordane concentration, µg/l | | | | | |
|---|---|---|---|---|---|---|
| | Tank 1 | Tank 2 | Tank 3 | Tank 4 | Tank 5 | Tank 6 |
| Test 1 | | | | | | |
| 5/3/74 | 0[a] | 1.1 | 3.4 | ... | 25.9 | 48.0 |
| 5/7/74 | 0 | 0.9 | 1.8 | 4.6 | 9.8 | 15.2 |
| 5/17/74 | 0 | 1.0 | 2.0 | 3.6 | 7.1 | 10.2 |
| Mean | 0 | 1.0 | 2.4 | 4.1 | 14.3 | 24.9 |
| Standard deviation | ... | 0.1 | 0.9 | 0.7 | 10.2 | 16.8 |
| Test 2 | | | | | | |
| 6/6/74 | 0 | 0.5 | 0.9 | 2.9 | 5.6 | 11.8 |
| 6/11/74 | 0 | 0.8 | 2.2 | 3.3 | 6.9 | 15.6 |
| 6/24/74 | 0 | 0.8 | 2.0 | 3.6 | 9.5 | 19.2 |
| Mean | 0 | 0.7 | 1.7 | 3.3 | 7.3 | 15.5 |
| Standard deviation | ... | 0.1 | 0.7 | 0.4 | 2.0 | 3.7 |

[a] No technical chlordane detected.